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RESULTS OF ADMINISTRATION OF ANTERIOR PITUITARY ADRENOCORTICOTROPIC HORMONE TO A NORMAL HUMAN SUBJECT*†

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STUDIES on animals have shown that purified adrenocorticotropic hormone (ACTH) of the anterior lobe of the pituitary body is able to stimulate the adrenal cortex of the intact or hypophysectomized animal to increased function and produce results similar to those that have been produced by administration of crystalline adrenocortical hormones (2, 11, 14, 16, 28, 39, 40, 41, 51, 52). The primary aim of this study was to determine what changes might occur in the excretion of urinary steroids as the result of administration of ACTH to a normal human subject. Observations also were made on many other factors that are known to be influenced by activity of the adrenal cortex.

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It is well known that most patients with tumors or hyperplasia of the adrenal cortex excrete abnormally large amounts of 17-ketosteroids in the urine (9, 15, 36). Under conditions which presumably stimulate the adrenal cortex to increased activity, an increased excretion of material with chemical and physiologic properties similar to those of the 11-oxygenated hormones of the cortex has been observed (6, 50). Relatively large amounts of pregnanediol have been found in the urine in cases in which hyperfunction of the adrenal cortex has been associated with an absence of ovarian function (33). Estrone has been isolated from extracts of the adrenal gland (1) and in a few instances, large amounts of estrogens have been found in the urine in cases of tumor of the adrenal cortex (19, 20, 33). These observations suggested that stimulation of the adrenal cortex with ACTH would result in an increase in the excretion of 17-ketosteroids and cortin-like material and possibly in the excretion of increased amounts of estrogen and pregnanediol. Determinations of the quantitative variations in these urinary steroids were made and, so far as it was possible to do so by isolation procedures, the qualitative variations were studied.

In Cushing's syndrome, which is believed to result from hyperfunction of the adrenal cortex, the following changes in the blood have been observed: an increase in the concentration of sodium and bicarbonate in the plasma (54), a decrease in the concentration of potassium and chloride in the plasma, and polycythemia (12). The following changes have been observed after the administration of adrenocorticotropic hormone to animals: an involution of lymphoid tissue, a decrease in the number of circulating lymphocytes (11, 14, 41), a polymorphonuclear leukocytosis, an increase in the β-globulin and γ-globulin fractions of the plasma proteins (53), an increased excretion of nitrogen (22, 24), and, under some circumstances, a glycosuria (26). Many of these effects have been produced in animals by administration of crystalline adrenocortical hormones (25).

An increase in the production of the 11-oxygenated hormones of the adrenal cortex might be expected to cause changes in carbohydrate metabolism which would result in a decreased tolerance for glucose and an increased resistance to insulin (23). An increased production of compounds like desoxycorticosterone would be expected to cause a retention of sodium, chloride and bicarbonate and an increased excretion of potassium (45).

METHOD OF STUDY

In order to avoid possible complications by testicular steroids, a woman was chosen as the subject for this study. The subject was a white, single woman who was thirty-two years of age and weighed 68 Kg. She was in good health; she had not had any serious illness and had not undergone an operation. Her menstrual periods were regular. She was maintained on a

constant diet in a unit designed for metabolic studies. She was observed for thirty-three days before the administration of the hormone was started.

The adrenocorticotropic hormone was administered subcutaneously in five equal doses between 7:30 a.m. and 11:30 p.m. The initial daily dose was 25 mg. Since a definite effect was not observed during the first six days, the daily dose was increased to 50 mg. for six days and then, after an interval of five days, to 100 mg. This dose was administered for 11.5 days.

The hormone was prepared by the procedure of Li, Evans and Simpson (29). It was dissolved in a 0.9 per cent solution of sodium chloride. The first preparation, which contained 5 mg. of the hormone per milliliter, was sterilized by passage through a Berkefeld filter. Since there appeared to be some loss of protein on the filter, subsequent preparations were heated one hour in a boiling water bath. The concentration of the hormone was increased to 10 mg. per milliliter. The solution of ACTH was stored in the refrigerator when not in use.

The amount of 17-ketosteroids excreted in the urine was determined by the method of Callow, Callow and Emmens (10) and by application of the correction equation of Talbot, Berman and MacLachlan (43). The urinary steroids were isolated by the procedures described by Mason and Kepler (33). The amount of cortin-like substances was determined in the following manner. The urine was extracted and the extract was washed according to the procedure of Talbot, Saltzman, Wixom and Wolfe (44). The crude extract was separated into ketonic and nonketonic fractions with the aid of Girard's reagent T. The amount of α-ketol in the ketonic fraction was determined essentially by the method described by Lowenstein, Corcoran and Page (31).

The value for the pregnanediol excreted in the urine was determined by the method of Venning (49), the value for estrogen* was determined by the procedure of Smith and Smith (42) and the value for the gonadotropin was determined by the method of Frank (21).

The glucose tolerance test was performed in the following manner. After a specimen of blood had been removed for determination of the value for the blood sugar, 0.1 Gm. of D-glucose per Kg. of body weight was administered intravenously, in the form of a 20 per cent solution, over a period of thirty minutes. Subsequent determinations of the concentration of blood sugar were made at intervals of thirty, sixty, ninety, a hundred and twenty, a hundred and fifty, and a hundred and eighty minutes after completion of the injection.

The following technic was used in performing the insulin tolerance test.

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* We are indebted to Dr. Alexander Albert for the assays of urinary estrogen and gonadotropin.

After a specimen of blood had been removed for determination of the value for the blood sugar, 0.1 unit of regular insulin per Kg. of body weight was injected intravenously. The coneentration of the blood sugar was determined at intervals of twenty, thirty, forty-five, sixty, ninety and a hundred and twenty minutes after the injection.

The values for the electrolytes, creatine, creatinine and total nitrogen in the blood and urine were determined by standard methods. The carbon dioxide content of plasma collected under oil was determined by the method of Van Slyke and Neill (48). The values for the plasma cholesterol and cholesterol esters were determined by the methods of Bloor (3), and Bloor and Knudson (4), respectively. The value for the ascorbic acid was determined by the method of Pijoan and Klemperer (37). The value for the serum protein and the albumin-globulin ratio were determined by Kingsley's (27) method. The value for the blood urea was determined by Marshall's method as modified by Van Slyke and Cullen (47). The value for the blood sugar was determined by the method of Folin and Wu (18); that for the serum alkaline phosphatase was determined by Bodansky's (5) method and that for the serum uric acid was determined by Folin's method (17).

RESULTS AND COMMENT

The injections of ACTH caused a local reaction which consisted of redness, burning and itching followed by soreness. After a few days the redness was replaced by a brownish discoloration which persisted for a month or two. When the daily dose of the hormone was increased to 100 mg., the subject became pale, listless and apathetic, and complained of generalized malaise, and of vague aches and pains which resembled those which occur during the onset of influenza. These symptoms disappeared after three days. About this time, a mild pitting edema occurred in the ankles and in the pretibial region, the weight increased to 69.5 Kg. and severe acne appeared on the face. After administration of the hormone was discontinued, the extra weight was lost rapidly and the edema and acne began to disappear. The acne, however, disappeared very slowly and was still evident one month later.

The hormone had a most striking effect on the urinary excretion of 17-ketosteroids and cortin-like material (Table 1). For isolation of steroids, urine was collected for thirty-nine days before the subject was placed under the controlled conditions of the metabolic unit. Five determinations of the 17-ketosteroids were made during this period. The results of these determinations are included in the average values for the control period. During the control period, seven twenty-four hour specimens were used for determining the value for the 17-ketosteroids.

In the first two periods during which ACTH was administered there

TABLE 1. EFFECT OF SUBCUTANEOUS ADMINISTRATION OF ADRENOCORTICOTROPIC HORMONE ON THE URINARY EXCRETION OF 17-KETOSTEROIDS AND CORTIN-LIKE SUBSTANCES

Period of Study		ACTH, mg. Administered Per Day	17-Ketosteroids, mg. Excreted Per Day	Cortin-like Substances, mg. Excreted Per Day
Thirty-three days* (control period)		None	4.84 (3.9–6.2)†	0.180 (0.174–0.186)†
Six days		25	5.62 (4.9–6.6)†	0.255 (0.229–0.274)†
Six days		50	5.95 (4.0–7.3)†	0.387 (0.282–0.450)†
Five days		None	6.6 (4.8–8.5)†	0.243 (0.232–0.252)†
Twelve days	1st day	100	10.5	0.556
	2nd day	100	10.7	0.577
	4th day	100		0.952
	6th day	100	13.2	1.090
	7th day	100	12.4	1.050
	8th day	100	15.5	1.440
	10th day	100	15.5	1.020
	12th day	50‡	11.6	0.797
Six days	1st day	None	5.3	0.105
	2nd day	None	4.8	0.166
	3rd day	None	3.2	0.179
	6th day	None	4.0	0.199

* The excretion of 17-ketosteroids was determined for an additional period of thirty-nine days.

† Range of values.

‡ Amount administered during first half of day.

appeared to be a small increase in the average value of the 17-ketosteroids but the change was too small to be significant particularly when the values were compared with those obtained in the five day interval between the periods during which 50 mg. and 100 mg. of the hormone were administered. Administration of 100 mg. of ACTH was necessary to produce an unequivocal increase in the output of the 17-ketosteroids. The peak value of 15.5 mg. was more than three times the average control value.

The excretion of cortin-like substances increased significantly even dur-

ing the period when the smallest amount of the hormone was administered. The amount of cortin-like substances finally reached a peak of 1.44 mg., which was eight times the average control value. The immediate decrease which occurred in the excretion of 17-ketosteroids and cortin-like substances when administration of the hormone was discontinued is noteworthy. Even the decrease in the amount of hormone to 50 mg. on the twelfth day of the last period of administration of the hormone resulted in a corresponding decrease in the excretion of these steroids.

Pregnanediol was not present in the urine in determinable amounts

TABLE 2. URINARY EXCRETION OF STEROIDS BEFORE AND DURING THE ADMINISTRATION OF ADRENOCORTICOTROPIC HORMONE

Steroids Isolated	Amount Excreted			
	During Control Period of 72 Days		During First 30 Days After Administration of Hormone Was Started	
	Total Weight, mg.	Mg. Excreted Per Day	Total Weight, mg.	Mg. Excreted Per Day
Androsterone	19.5	0.27	32.0	1.06
Etiocholanolone	7.0	0.10	32.0	1.06
Pregnanediol	34.0	0.47	14.0	0.46
Cholesterol	41.0	0.57	7.0	0.23

during the first part of the menstrual cycle in either the control period or the period when 100 mg. of ACTH was administered. In the latter part of the cycle, a maximum of 7 mg. was found during both periods. Evidently the adrenal cortex was not stimulated to produce a sufficient amount of progesterone or other precursors of pregnanediol to permit detection of the latter substance in the urine when the ovarian production of progesterone was nil.

The excretion of estrogen and gonadotropin remained within the normal range throughout the study.

Isolation of the urinary steroids gave the results summarized in Table 2. It will be noted that the amounts of androsterone and etiocholanolone that could be isolated, calculated as milligrams per day, increased approximately four and ten times, respectively, whereas the amount of pregnanediol remained unchanged. The significance of the presence of cholesterol in the urine is obscure. The decreased amount that could be isolated during the period when ACTH was administered may possibly be correlated with

the decreased concentration of cholesterol in the plasma. A careful search was made for 11-hydroxyandrosterone. This substance has been obtained in relatively large amounts from the urine of patients with hyperfunctioning lesions of the adrenal cortex (33, 35) and the appearance of this substance in the urine or an increase in the amount previously present would have been considered additional evidence of an increased production of 11-oxygenated steroids by the adrenal cortex under the stimulus of ACTH. It could not be found either before or after administration of the hormone. Likewise, dehydroisoandrosterone could not be found in either period. Digitonin failed to give a precipitate with the ketonic fractions of the extracts and also with the eluates of the chromatographic column which preceded the eluates containing androsterone. Dehydroisoandrosterone is eluted just before androsterone and sometimes several fractions are mixtures of the two substances. Dehydroisoandrosterone is considered to be a product of the adrenal cortex since it often appears in the urine in large amounts in cases of adrenocortical tumor (33). However, it is readily converted to androsterone and etiocholanolone (32, 34). If an increased production of this substance occurred as the result of stimulation of the cortex by ACTH, it was not sufficient to permit it to appear in the urine in detectable amounts.

Thus, as far as could be determined by means of isolation, the qualitative pattern of the urinary steroids remained unchanged during administration of ACTH. The change in the quantitative relation between androsterone and etiocholanolone is interesting. It is generally considered that normally these two substances are excreted in approximately equal amounts (7, 8, 13). In this case, however, equal amounts were isolated only during the period when ACTH was administered. It may be that this result was due to some unrecognized discrepancy in the procedures of hydrolysis, extraction and isolation.

The increased excretion of urinary 17-ketosteroids and cortin-like substances under the influence of ACTH followed by the abrupt return of the excretion to control levels when administration of the hormone was discontinued is considered to be unequivocal evidence that the adrenal cortices of this normal human subject were stimulated to a state of hyperfunction by the ACTH.

The chemical composition of the blood was changed very little during administration of 100 mg. of ACTH. There was no significant change in the concentration of sodium, potassium, chloride, phosphorus and nonprotein nitrogen. The carbon dioxide content of the plasma increased from 26.2 to 29.6 milliequivalents per liter as shown in Fig. 1. Although this change is not great it is believed to be significant since the greatest change occurred when the subject received 100 mg. of ACTH and since the carbon dioxide

content of the plasma decreased again after administration of the hormone was discontinued.

The concentration of plasma proteins, the albumin-globulin ratio, and the electrophoretic pattern of the proteins remained essentially unchanged. The value for the serum alkaline phosphatase remained within normal limits in contrast with the decrease which Li, Kalman, Evans and Simpson (30) found in young rats treated with ACTH. The vitamin C content of the plasma varied between 1.2 and 1.5 mg. per 100 ml. during the periods of control and of administration of ACTH. The value for the uric acid of the serum also was not affected by administration of ACTH.

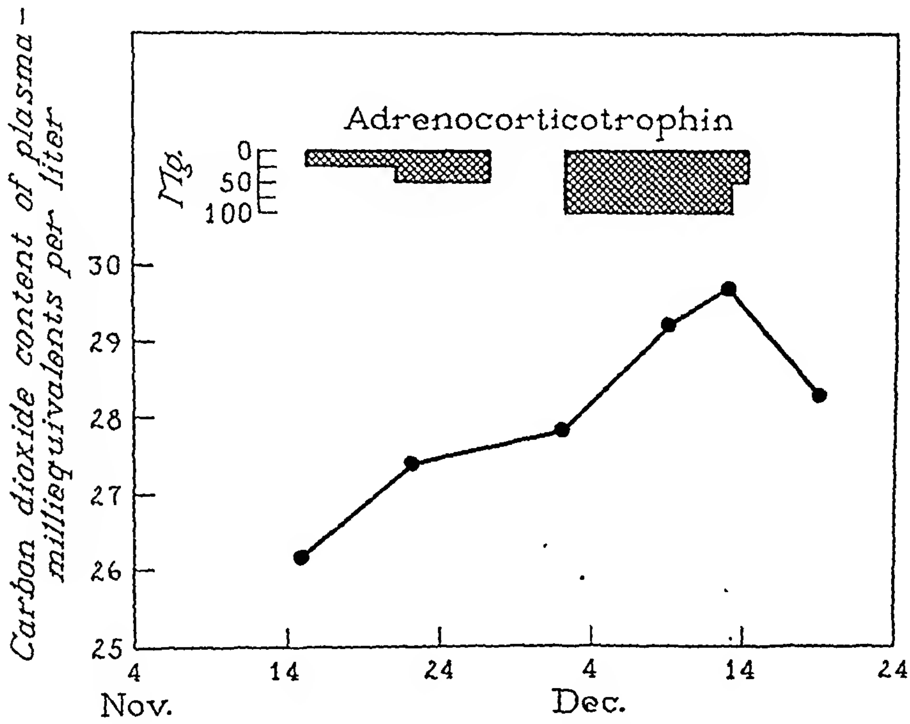

Fig. 1. Effect of ACTH on the carbon dioxide content of plasma.

The concentration of plasma cholesterol appeared to be affected by ACTH as shown in Table 3. It will be observed that the decrease occurred largely in the free cholesterol and that the values returned to the control level within six days after administration of the hormone was discontinued (last dose administered on December 13). An attractive suggestion is that the increased production of adrenal steroids resulted in withdrawal of cholesterol from the plasma for this purpose. This suggestion, of course, arises from the observation that stimulation of the adrenal cortex of animals with ACTH results in a prompt decrease in its content of cholesterol (38). Presumably, the supply is replenished from the plasma.

Dougherty and White (14) found that ACTH produced a lymphopenia and a polymorphonuclear leukocytosis in rats. Thorn, Prunty and Forsham (46) have recently reported similar observations in a man with mild pituitary insufficiency who was treated with ACTH. Such an effect was not obtained with our normal subject until she had received the hormone for twenty-two days. Even then, this effect was not observed until after the second dose of the day had been administered and the effect lasted less than three hours. In view of the previous lack of effect, it seems questionable that this single result can be attributed to the action of ACTH.

TABLE 3. EFFECT OF SUBCUTANEOUS ADMINISTRATION OF ADRENOCORTICOTROPIC HORMONE ON THE CONCENTRATION OF PLASMA CHOLESTEROL

Date, 1946	ACTH, mg. Administered Per Day	Cholesterol, mg. Per 100 cc. of Plasma	
		Total Cholesterol	Cholesterol Esters
Oct. 30	None	247	127
Nov. 15	None	240	
Nov. 16	25	247	142
Nov. 22	50	190	147
Dec. 2	None	217	133
Dec. 9	100	197	130
Dec. 13	100	217	139
Dec. 19	None	247	141

An anemia developed rapidly during the period when the daily dose of ACTH was 100 mg. The value for the hemoglobin fell from 11.5 Gm. to 8.55 Gm. per 100 ml. and the cell volume per cent fell from 37 to 30. The erythrocyte count remained quite constant. These results again are in disagreement with those of White and Dougherty (52) who observed an elevation of the erythrocyte count and of the concentration of hemoglobin after prolonged administration of ACTH to rats. These results also are contrary to those expected from observations of patients with hyperfunctioning lesions of the adrenal cortex.

The urinary excretion of sodium, potassium and chloride was not affected by administration of ACTH. There was possibly an increased excretion of nitrogen during the last seven days of the period when 100 mg. of the hormone was administered. During this time the average daily excretion of nitrogen was 10.85 Gm. (10.1 to 11.5 Gm.) but on three days of the control period the average amount of nitrogen excreted daily was

10.61 Gm. (10.17 to 10.87 Gm.). Consequently, it is not certain that the increased excretion of nitrogen was caused by the ACTH although the over-all average daily excretion during the control period was 9.23 Gm.

The excretion of creatinine nitrogen remained quite constant throughout the period of study. The values for the urinary creatine nitrogen, however, fluctuated widely. During the control period, between 48 and 201 mg. of creatine nitrogen were excreted per day. During the latter ten days of the period when 100 mg. of ACTH was administered, the range of values was 13 to 77 mg. and the average value was 43 mg. During five days immedi-

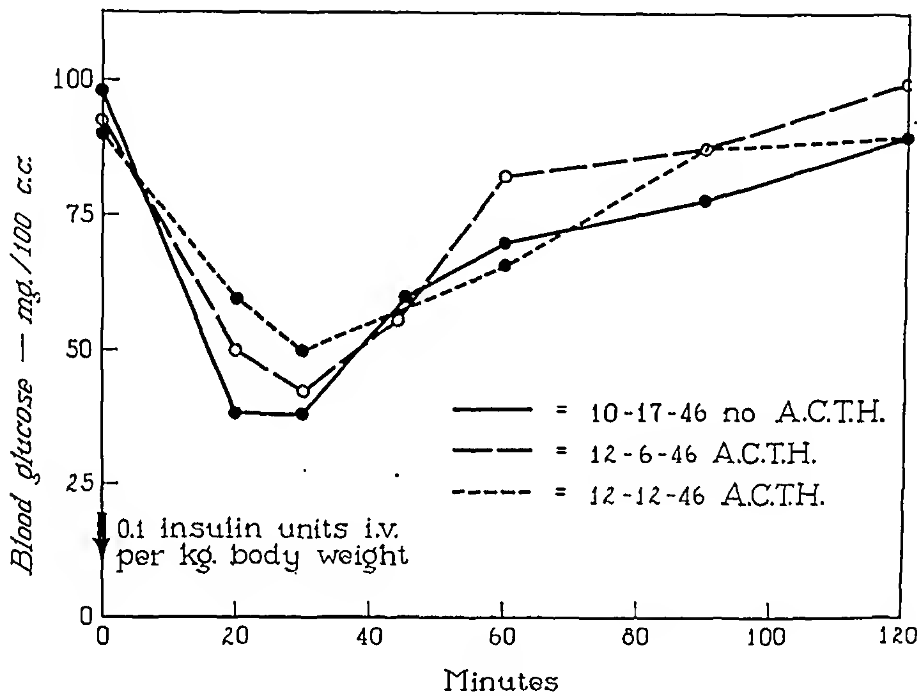

FIG. 2. Blood sugar—time curves after a standard dose of insulin.

ately after administration of the hormone was discontinued, the values ranged from 49 to 96 mg. and the average value was 71 mg. It appears that there was a definite decrease in the excretion of creatine nitrogen during administration of 100 mg. of ACTH. The appearance of acne during this period suggests that an extra amount of adrenal androgens was produced. Perhaps there was sufficient androgenic effect to influence the excretion of creatine.

The measurable effect of the hormone on carbohydrate metabolism was not marked. The glucose tolerance test did not reveal any decreased tolerance but the results of the insulin tolerance test suggested an increased resistance to insulin as the result of administration of the hormone. The blood sugar curves after injection of insulin are shown in Fig. 2.

SUMMARY

When 100 mg. of the anterior hypophyseal adrenocorticotropic hormone was administered daily, in divided doses, to a normal young woman it caused an increased excretion of urinary steroids, which is evidence of stimulation of adrenocortical function. The daily excretion of 17-ketosteroids increased from 4.84 to 15.5 mg. and that of the cortin-like substances increased from 0.180 to 1.44 mg. Development of acne and a decrease in the excretion of creatine suggested that the production of androgens was increased. The excretion of pregnanediol, estrogens and gonadotropin was not affected measurably. Androsterone and etiocholanolone were isolated from the urine in increased amounts during the period when ACTH was being administered. The amount of pregnanediol that could be isolated was unchanged by administration of the hormone. The amount of cholesterol that was isolated from the urine was decreased during the period of administration of the hormone.

Electrolyte metabolism appeared to be essentially undisturbed by the hormone as judged by the concentration of sodium, potassium, chloride and phosphorus in the blood and urine. There was a small but apparently significant increase in the carbon dioxide content of the plasma.

The value for the urinary nitrogen increased but the increase was so small that it could not be attributed with certainty to administration of the hormone.

The value for the free cholesterol of the plasma decreased significantly. There was no change in the quantity of the blood proteins or in their quality as determined by electrophoresis. The value for the serum alkaline phosphatase remained unchanged. Anemia developed but the erythrocyte and leukocyte counts were not affected significantly.

Tolerance to glucose was not affected measurably but some resistance to insulin developed.

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CLINICAL STUDIES WITH PITUITARY ADRENOCORTICOTROPIN

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INTRODUCTION

In 1924 Evans (25) reported that the injection of anterior pituitary extract was followed by adrenal hypertrophy. Smith (61, 62) in 1926 demonstrated that hypophysectomy induced atrophy of the adrenal cortex and that implantation of living hypophyseal tissue was followed by restoration of the gland to normal. These classical experiments established without doubt the specific tropic influence of the anterior pituitary on adrenal cortical function.

Isolation of the adrenocorticotropic principle from anterior pituitary gland extract was reported by Collip in 1933 (15). In 1940 a somewhat simpler mode of preparation was described by Bates *et al.* (8). Further purification of the adrenocorticotropic hormone was accomplished by Evans and his group in California and Long and Sayers and their collaborators in New Haven. The former group worked with sheep pituitaries; whereas the latter used hog pituitaries. In 1943 Li *et al.* (41), employing a salt fractionation method, and Sayers *et al.* (56), using isoelectric precipitation, published detailed accounts of their methods of preparing pure adrenocorticotropin. Although this particular hormone was derived from pituitaries of different species and prepared by different methods in the two laboratories, the preparations appeared to be identical by the criteria employed.

Purified anterior pituitary adrenocorticotropin proved to be a protein molecule with a single peak in the electrophoretic pattern, an isoelectric point at pH 4.7, and a molecular weight of approximately 20,000. That the activity of the preparation might reside in a smaller unit is suggested by the studies of Li *et al.* (40, 41), Tyslowitz (75), and Crooke *et al.* (18).

The preparation of small quantities of purified pituitary adrenocorticotropic hormone (ACTH) with a high degree of potency permitted more precise physiological studies in animals. Experiments indicated that the injection of ACTH led to increased secretory activity of the adrenal cortical cells and eventually to their hypertrophy (60). The effects which were

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observed to follow the injection of ACTH appeared to be due to the elaboration of the adrenal cortical steroids rather than to a specific effect of ACTH itself.

The minute quantities of purified ACTH available prior to 1946 precluded clinical trial with the exception of a single experiment carried out by Browne *et al.* in 1943 (11) in which purified ACTH was administered to two male patients. In one subject these authors observed an increased urinary excretion of "cortin-like" substances without a significant change in the output of 17-ketosteroids. In a second patient an increase in the height of the glucose tolerance curve and glycosuria followed ACTH administration. From these preliminary experiments it appeared that the principal action of ACTH on the human adrenal was the stimulation of 11-oxysteroid secretion (the so-called carbohydrate-regulating factors).

From what is known of adrenal cortical function in man, however, one might anticipate that with a gland capable of responding to ACTH therapy it would be possible to demonstrate the several actions of the three types of adrenal steroids, viz.,

1. The sodium and chloride-retaining effect of desoxycorticosterone-like steroids (67)
2. The gluconeogenetic (44) and lympholytic (21) activity of the 11 and 11-17-oxysteroids
3. The nitrogen-retaining effect of androgenic adrenal steroids (1)

Recently the Armour Laboratories have prepared a quantity of purified ACTH adequate for clinical trial.[1] With this material we have attempted to answer the following questions:

1. What are the over-all metabolic and hematologic effects of ACTH administration in man?
2. Is there a readily measured response to ACTH which might provide a satisfactory clinical evaluation of adrenal cortical reserve?

MATERIALS

The pituitary adrenocorticotropic hormone (ACTH) used in these studies was prepared by the Armour Laboratories from hog pituitary glands by a modification of the method of Sayers et al. (56), using isoelectric precipitation in the cold following an acid acetone extraction. Four different batches of hormone (ACTH) were placed at our disposal during the course of these studies. Batch 13-10, after preliminary experiments, was returned for further purification because of an unduly high content of posterior pituitary factor which seriously restricted the quantity of material which could be administered without inducing undesirable side effects. Batches

[1] The authors are indebted to Dr. John R. Mote, The Armour Laboratories, Chicago, Illinois, for the purified ACTH used in these studies.

21-B and 13-10-6 were used extensively; a few patients received material from Batch 32-D. The characterization of the several batches is summarized in Table 1 from data provided through the courtesy of Doctors Munson, Koch, and Thompson and their staffs of the Armour Laboratories.

Although the method of preparing the ACTH effectively excluded appreciable contamination with certain anterior pituitary hormones, i.e. thyrotropic, gonadotropic, and growth hormones, the material used in these studies could not be considered to be pure adrenocorticotropin. None of the preparations employed showed a single electrophoretic peak. The potency of the batches as measured by the decrease in ascorbic acid content of the rat adrenal (method of Sayers (55)) is summarized in Table 1. In addition, batches 13-10-6 and 21-B were administered to male and female hypophysectomized rats. Adrenal hypertrophy occurred in both groups of animals and was associated with a decrease in testicular weight in the males and no significant change in weight of the ovaries, thyroid, or uterus in the females. These data suggest that the batches used almost exclusively in these clinical studies were not appreciably contaminated with thyrotropic or gonadotropic activity. The low lactogenic activity of the ACTH is indicated in Table 1. The oxytocic and pressor activity varied consider-

TABLE 1. PROPERTIES OF THE ACTH PREPARATIONS EMPLOYED

Batch	*ACTH Activity* Per Cent of Armour Standard Preparation*	*Pressor Activity* Rooster B.P. Method Units/mg.	*Oxytocic Activity* Guinea Pig Uterine Strip Method Units/mg.	*Prolactin Activity* Pigeon Crop Sac Method Units/mg.	Solubility
21-B	102 ±8	0.1–0.2	0.12–0.004	0.5	Soluble in alkaline saline *p*H 8
13-10-6	83 ±9	0.07 or less	0.05 or less	0.25 or less	Soluble in saline
32-D	71 ±9	0.10	0.05	1.8 ±15	Soluble in acid saline *p*H 2–3
13-10	83 ±5	0.7	0.6	—	Soluble in saline

* 0.4 gamma of the reference standard (Armour La-1-A) administered intravenously to hypophysectomized rats induces a 20 to 30 per cent decrease in adrenal ascorbic acid content by the method of Sayers and Sayers (55).

ably from batch to batch (Table 1). Unfortunately, comparable assays of the antidiuretic effect were not made.

Preparation for Clinical Use

Sterilization by ultrafiltration was not attempted, since this procedure results in a loss of biologic activity. Therefore the material was handled with aseptic care throughout, dissolved in sterile saline, and adjusted to an appropriate pH. Since appreciable loss of activity occurred within twenty-four hours with the material in solution, *a fresh solution was made up daily, and the solution was never allowed to remain at room temperature for more than one hour.* ACTH powder and the frozen solution appeared to retain activity for months.

Administration

The ACTH was administered intramuscularly as a solution containing from 5 to 10 mg. per cc. in saline. The pH varied with the several preparations (Table 1). Apart from soreness, no local untoward reactions were observed in over two hundred and fifty injections. Depending upon the content of posterior pituitary factors, there was a variable degree of transitory pallor, bradycardia, intestinal and uterine cramps. These symptoms were negligible in the preparations with low oxytocic activity.

Steroid Preparations Used for Intramuscular Injection

Desoxycorticosterone Glucoside:[2] This preparation contained 10 mg. of crystalline hormone in aqueous solution as supplied by the manufacturer It was used in doses up to 30 mg.

Dehydrocorticosterone Hemisuccinate, Compound A:[3] This lyophilized crystalline material which was freely soluble in saline solution was administered in doses of from 25 to 50 mg. intramuscularly.

17-Hydroxycorticosterone, Compound F:[4] This crystalline material was dissolved in warm absolute alcohol (20 mg. per cc.) which was added to 4 cc. of 1 per cent acid procaine just prior to intramuscular injection in doses of 20 mg.

Testosterone-Diethyl-Amino-Ethyl-Carbonate-Hydrochloride:[2] This crystalline material, which is freely soluble in saline up to 10 mg. per cc., was used in quantities up to 35 mg.

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[2] The authors are indebted to Dr. Ernst Oppenheimer, Ciba Pharmaceutical Products, Inc., Summit, New Jersey, for the material used in these studies.

[3] The authors are indebted to Dr. Augustus Gibson, Merck & Co., Inc., Rahway, New Jersey, for the material used in these studies.

[4] The authors are indebted to Dr. M. H. Kuizenga, The Upjohn Company, Kalamazoo, Michigan, for the material used in these studies.

METHODS

All patients and subjects were maintained in the Metabolic Unit of the Peter Bent Brigham Hospital throughout the period of study. In the case of balance experiments, the patients and normal subjects were maintained on a constant diet. With the short-term experiments, patients were studied in the postprandial state, the last food being given at 8:00 p.m. on the day previous to the ACTH test.

Urine specimens were collected at appropriate intervals, and aliquots were stored after acidification and addition of a few drops of chloroform thymol preservative. When the excretion of 17-ketosteroids (12, 64) and 11-oxysteroids (66) was followed, the entire volume was saved. Particular care was taken to acidify the urine with 50 per cent sulfuric acid, and these specimens were frozen whenever immediate analysis was not possible and especially when uric acid and 11-oxysteroids were determined.

Stool specimens were collected in three-day periods and homogenized in a Waring blender, and aliquots were used for analysis.

Hematologic studies were made on venous blood specimens drawn with minimal stasis after a twelve-hour fast with the patient in basal condition. The blood was preserved in balanced oxalate[5] and stored in the icebox if analysis was delayed. Hematocrit (packed red cell volume) was estimated by the method of Wintrobe (78). White blood count and differentiation of cells were carried out by routine methods. Circulating eosinophil counts were performed by a modification of the direct method of Dunger (24), i.e.

Oxalated blood was drawn into a white count pipette to the 0.5 mark after which a special fluid[6] was used to complete the dilution to the 11 mark. The pipette was shaken at once for thirty seconds only; the counting chamber was filled immediately, and after waiting three minutes the eosinophils were counted. These cells may be identified by their deeply stained red granules. A special Levy chamber (0.2 mm. in depth) was used, and the average of four chamber counts was computed. The chamber count divided by sixteen and multiplied by one hundred yields the number of circulating eosinophils per cubic millimeter.

The following chemical methods were used in this study: Sodium in serum, urine, and diet aliquots was determined by the method of Con-

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[5] Balanced oxalate solution:

	Potassium oxalate	0.8 Gm.
	Ammonium oxalate	1.2 Gm.
	Aqua destillata q.s. ad	100.0 cc.

Five-tenths cc. of the solution is placed in a suitable container and evaporated. This amount of oxalate will prevent the coagulation of 5 cc. of blood.

[6]

Eosin aqueous, 2 per cent	5 cc.
Acetone	5 cc.
Aqua destillata q.s. ad	90 cc.

The diluting fluid should be stored in an icebox, filtered before use, and prepared fresh every two weeks.

solazio and Dill (16), and urine potassium by the method of Consolazio and Talbott (17). In the case of patient J. W., however, all analyses of sodium and potassium were carried out on a flame photometer with an internal lithium standard, as were the urinary potassium determinations[7] in subject R.P. (10). Serum and urine chloride was measured by the mercurometric titration method of Schales (57) with the precautions recommended by Asper (5). The open Carius method was used for diet and stool specimens (52). Inorganic phosphorus in serum and urine was determined by the colorimetric method of Fiske and Subbarow (26), diet specimens being analyzed by the method of Allen (2). Urinary calcium was measured by the method of Clark and Collip (13). Serum carbon dioxide combining power was determined by the Van Slyke manometric technique (50). Total nitrogen in urine and total serum protein were measured by the microkjeldahl distillation method of Keys (37); total nitrogen in diet and stool specimens by a modified macrokjeldahl procedure suggested by Van Slyke (76); albumin and globulin by the method of Howe (34); blood nonprotein nitrogen by a modification of the method of Daly (19); blood urea nitrogen by the method of Archibald (4); and urine urea nitrogen by the hypobromite method of Van Slyke (51). Cholesterol, free and total, was analyzed by the modification of the method of Schoenheimer and Sperry (58), and blood sugars by the method of Folin and Wu (28), using Somogyi's zinc precipitation (63). Urinary creatine was determined by the method of Lambert (38), and urinary creatinine by the method of Folin (29). The method used for urinary and serum uric acid[8] in this study is presented in detail:

Preparation of Reagents

Stock standard uric acid solution containing 1 mg. per cc.: Prepared according to the method of Folin (27). From this a daily "working standard" is made up by diluting 1 cc. up to 200 cc. with distilled water.

Two per cent polyanethol sodium sulfonate (Liquoid, Hoffman-La Roche): One Gm. is dissolved in 50 cc. of distilled water and stored in the icebox.

Silicate glycerine reagent: Mix 130 cc. of Merck's waterglass with approximately 250 cc. of distilled water and add 85 cc. of reagent grade glycerol. Shake and make up to 500 cc. with water. If cloudy, filter through hard filter paper. Store in a pyrex container.

Uric acid reagent: Fifty Gm. of reagent grade sodium tungstate (free of chloride and neutral to phenolphthalein) is refluxed gently for at least four hours with 400 cc. of distilled water and 40 cc. of syrupy orthophosphoric acid, cooled, and made up to 500 cc. with water.

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[7] The authors are indebted to Dr. William M. Wallace for carrying out the flame photometer analyses.

[8] The authors are indebted to Dr. Reginald M. Archibald for the details of his modification of the Kern and Stransky (36) uric acid method.

Special reagent: This is made up about every two weeks by mixing five parts of 2 per cent Liquoid, four parts of uric acid reagent, and nine parts of distilled water.

Sodium hydroxide: Two per cent (0.5 N.)

Procedure

Preparatory: Plasma or *serum* (to be kept frozen if immediate analysis is not possible), 1 cc. is placed in a 25 cc. Erlenmeyer flask. Eight cc. of water and 0.4 cc. of 2 per cent sodium hydroxide are added, and the contents are mixed by rotation. Then 0.6 cc. of uric acid reagent is added slowly while the flask is shaken constantly. Contents of the flask are filtered through Whatman No. 42 filter paper after standing for five minutes. *Urine* (to be kept frozen if immediate analysis is not possible), 1 cc. is diluted to 100 or 200 cc. with distilled water, the higher dilution being preferable with specific gravities above 1.020. The material should be processed within one half hour since it is unstable at room temperature.

Colorimetric: Five cc. of filtrate or diluted urine is put into a Klett colorimeter tube; 5 cc. of water for the blank and 5 cc. of working standard for the standard are set up similarly. To each tube 2.5 cc. of silicate glycerine reagent is added, and the contents are mixed by shaking. This is followed by 2 cc. of special reagent and quick mixing. After standing the tubes for fifteen minutes at room temperature, they are read with a 660 red filter with the blank set to zero on the Klett Summerson photoelectric colorimeter. (Any other apparatus can be adapted to this method.)

Calculation: Plasma or serum uric acid, mg. per cent equals $\frac{\text{reading unknown}}{\text{reading standard}} \times 5$.

Urine uric acid, mg. per cent equals $\frac{\text{reading unknown}}{\text{reading standard}} \times 100$, using a 1:200 dilution.

Specificity: In eight urines an average of 94 per cent of the color was found to be destroyed by uricase at pH 9.2 in phosphate buffer under oxygen.

OBSERVATIONS

EFFECT OF A SINGLE INTRAMUSCULAR INJECTION OF ACTH

General Effects

On the basis of preliminary experiments (70, 73) a dose of 25 mg. of ACTH was selected as providing a near-maximum response. This amount of hormone was well tolerated by most patients. Immediate blanching of the skin occurred with bradycardia, mild abdominal cramps, and uterine cramps in females. These effects usually wore off completely in forty-five minutes and were noted in patients with Addison's disease as well as in normal subjects. It appeared that these reactions were due to the posterior pituitary contaminants, since clinical symptoms paralleled the oxytocic pressor assay of the several preparations used. As far as could be detected there was no symptomatic change in patients or in normal subjects at the height of the ACTH response three to five hours after the injection. Three female patients who had not menstruated previously for at least one year noted menstrual flow six to ten days after the injection of a single dose of 25 mg. of ACTH. One of these patients had Addison's disease.

Hematologic Changes

As previously reported by Hills *et al.* (33), the injection of 25 mg. of ACTH in patients with intact adrenals is followed by a fall in circulating

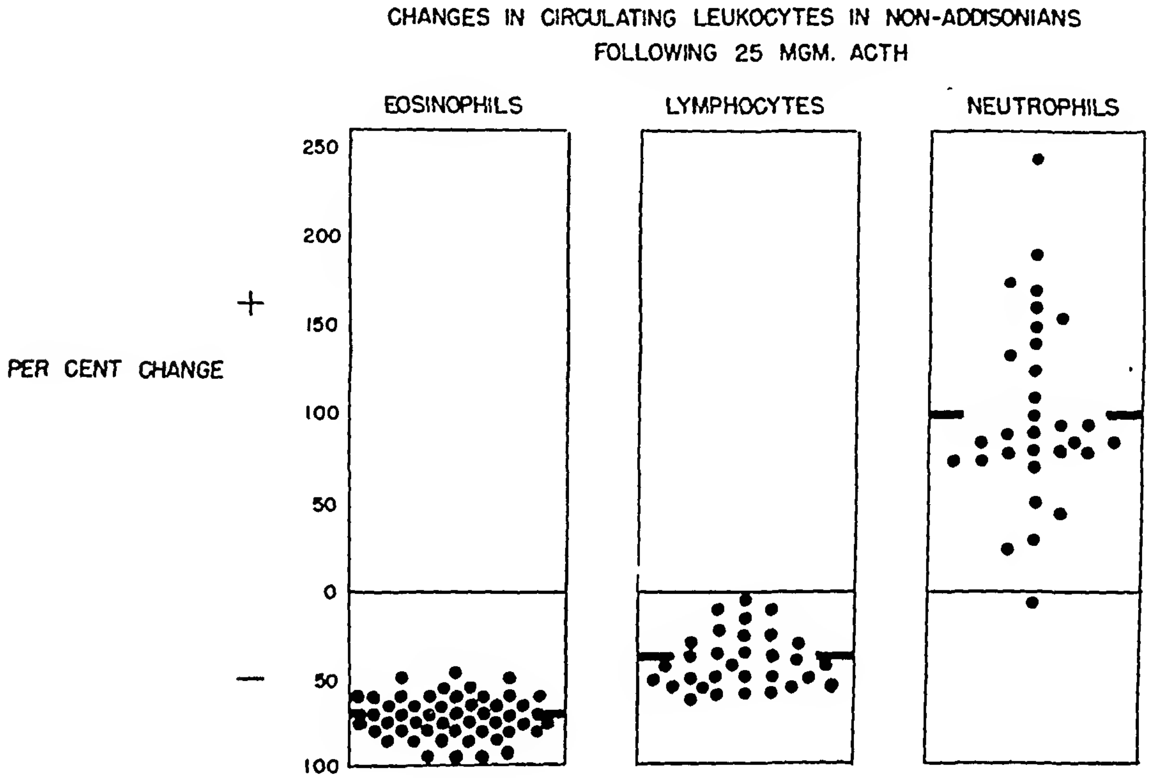

FIG. 1. Each point represents the result obtained on one subject. The heavy horizontal bars mark the mean value for the group.

TABLE 2. A COMPARISON BETWEEN THE ABSOLUTE REDUCTION AND THE PERCENTILE FALL IN CIRCULATING EOSINOPHILS FOLLOWING THE ADMINISTRATION OF 25 MG. OF ACTH

Patient	Sex	Age	Eosinophils per cu. mm.			Change
			8:00 a.m.	12:00 n.	Actual Decrease	
F.G.	F	18	21	4	17	−81%
A.D.	M	52	108	33	75	−70%
P.L.	M	24	288	61	227	−79%
R.F.	M	46	384	119	265	−69%

lymphocytes and eosinophils and a rise in polymorphonuclear leukocytes (Fig. 1). Changes in differential leukocyte counts resulting from administration of ACTH were expressed as a per cent of the preinjection counts.

This was based on the finding that the effect of a standard dose on the eosinophil count of normal subjects was quite uniform when expressed in this way irrespective of rather wide variations in the magnitudes of the initial counts (Table 2). Since the hematologic effects following a single intramuscular injection of ACTH were most pronounced at the end of four

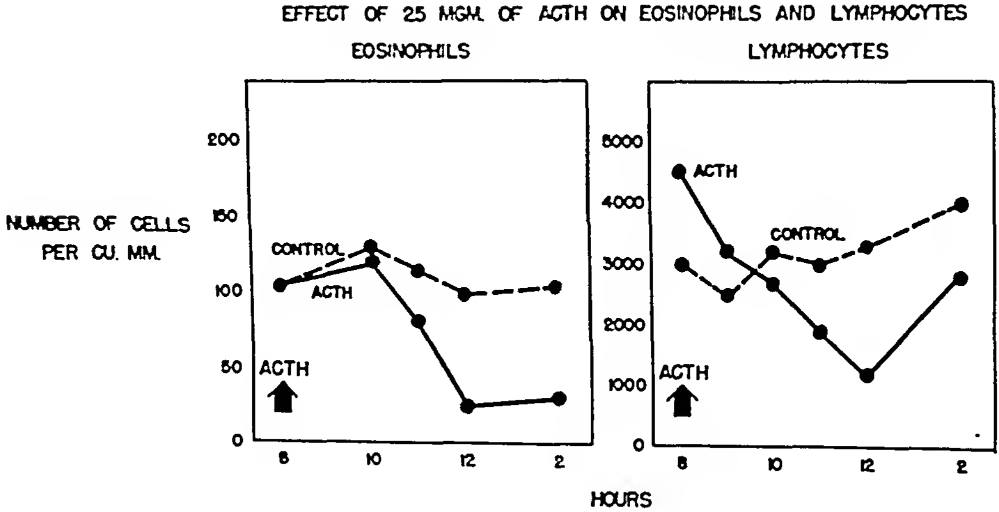

FIG. 2

TABLE 3. CHANGES IN EOSINOPHILS FOLLOWING ACTH ADMINISTRATION

Group	Number of Subjects	Eosinophil Count Cells per cu. mm. Mean Value for the Group		Change	Range
		8:00 a.m. Control	4 hrs. following 25 mg. ACTH i.m.		
Normal subjects	10	167	40	−77%	−63 to −97
Non-Addisonians	40	181	57	−73%	−52 to −98
Addisonians	30	247	235	− 4%	+36 to −38

hours (Fig. 2), this point was selected for measuring the maximum change in circulating leukocytes.

In contrast to the response of normal subjects and patients with adequate adrenal cortical function, patients with Addison's disease failed to show the characteristic hematologic changes, particularly the decrease in

lymphocytes and eosinophils, following the administration of ACTH (Fig. 3). It thus appeared that the fall in eosinophils and lymphocytes was dependent upon an intact adrenal cortex. Since the mean percentile fall in eosinophils following ACTH administration was approximately twice as great as that observed in the case of lymphocytes and since there was no overlap in the eosinophil response between the Addisonian and non-

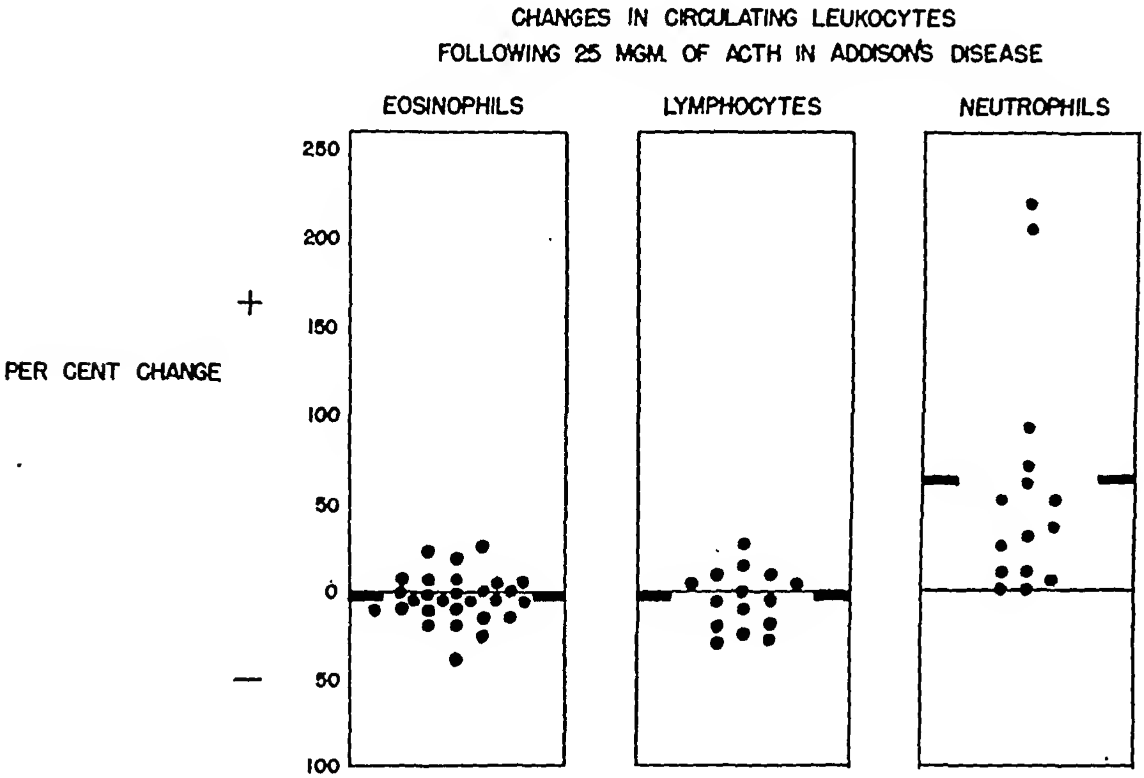

Fig. 3. Each point represents the result obtained on one subject. The heavy horizontal bars mark the mean value for the group.

Addisonian groups (Table 3, Figs. 1, 3), it must be concluded that the fall in circulating eosinophils is a more sensitive indicator of an increase in adrenal cortical hormone secretion than is the change in circulating lymphocytes.

To determine whether the failure of patients with Addison's disease to respond to ACTH was due to insufficient adrenal cortical hormone secretion rather than a peculiarity in leukocytes, 20 mg. of crystalline Compound F was administered to seven patients with Addison's disease. Since a fall in circulating eosinophils of 50 per cent or more was observed in six of the seven patients so treated (Table 4), the maximum effect occurring at four hours as was the case for non-Addisonians given ACTH (Fig. 4), it must be concluded that the failure to respond to ACTH was not due to an

abnormality of circulating leukocytes but rather to adrenal cortical hormone deficiency. The specificity of the 11-17-oxysteroid type of compound in producing these changes is suggested by the poor response of patients with Addison's disease to other types of water soluble steroids such as Compound A, desoxycorticosterone, and testosterone (Table 4).

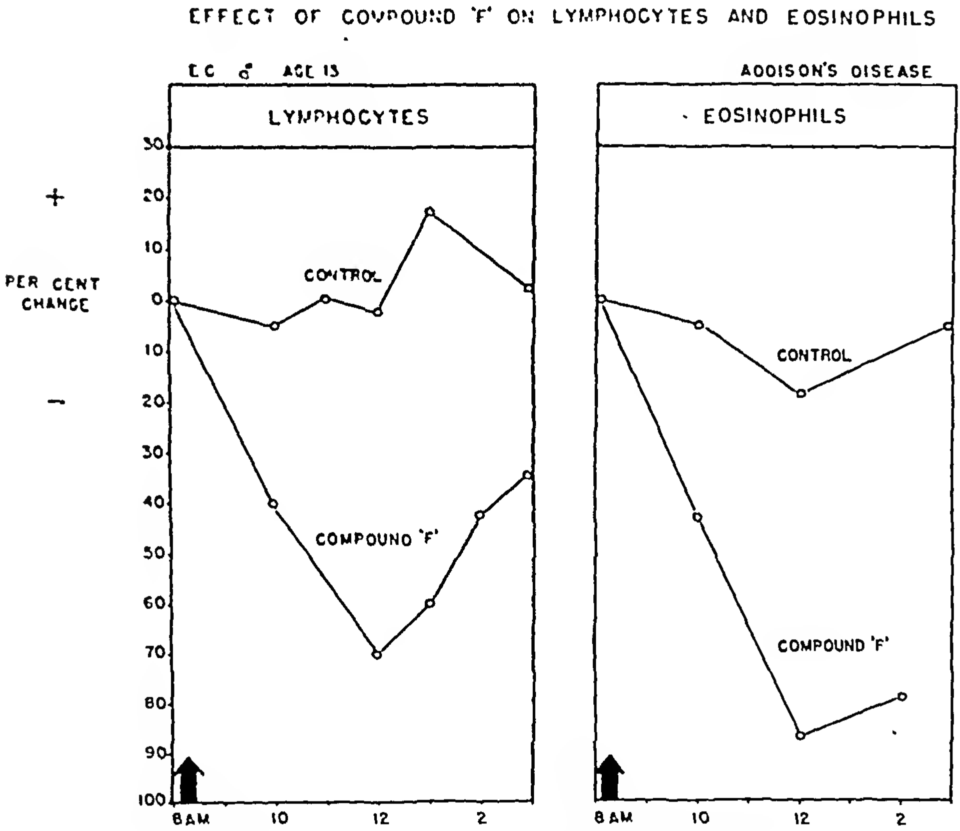

Fig. 4. Seventeen mg. of Compound F was administered intramuscularly at 8:00 a.m.

The failure of patients with Addison's disease to show a fall in the level of circulating eosinophils following ACTH administration indicated that neither ACTH itself nor contaminating anterior or posterior pituitary hormone in the ACTH preparation used in these studies was directly responsible for the hematologic changes. In patients with intact adrenals, however, one could not be certain that contaminating substances in the ACTH preparation did not contribute to the hematologic response by stimulating the output of anterior pituitary and adrenal cortical hormones. To test this possibility a dose of pitocin and pitressin ten times as large as

that contained in 25 mg. of ACTH was administered to three subjects who had responded to ACTH on a previous occasion. A fall in eosinophils of 40 per cent, 18 per cent, and 46 per cent respectively was observed. These studies suggest that it is possible for large quantities of posterior pituitary hormone, far exceeding those contained in the ACTH preparations, to in-

TABLE 4. EOSINOPHILIC RESPONSE TO THE INJECTION OF WATER SOLUBLE STEROID HORMONES IN PATIENTS WITH ADDISON'S DISEASE

Patient	Sex	Age	Hormone	Dose mg.	Control Level. Eosinophils per cu. mm.	Four Hours After Hormone Eosinophils per cu. mm.	Change
E.C.	M	13	17-hydroxy-corticosterone (Compound F)	17	570	75	−87%
J.P.	M	28		20	231	63	−75%
H.P.	F	33		20	157	55	−65%
M.N.	F	48		20	152	90	−65%
E.V.	M	49		20	486	188	−61%
H.S.	M	38		20	247	117	−53%
V.A.	F	38		20	2,300	1,810	−24%
J.B.	M	56	11-dehydrocorticosterone hemisuccinate (Compound A)	25	296	276	− 7%
E.C.	M	14		50	790	778	− 2%
C.S.	F	44	Desoxycorticosterone glucoside	30	208	225	+ 8%
J.G.	F	37		30	171	150	−12%
H.P.	F	33	Testosterone diethyl-amino ethyl-carbonate-hydrochloride	30	172	164	− 5%
E.Z.	F	58		35	84	86	− 2%

duce hematologic changes similar to those observed with ACTH. The magnitude of the response, however, never exceeded one-half that observed with 25 mg. of ACTH. These experiments, of course, failed to indicate whether the hematologic response was due to adrenotropic contaminant in the large dosage of posterior pituitary or whether the posterior pituitary extract in large quantities initiated an alarm reaction (59) with anterior pituitary and adrenal cortical hormone response.

The initial eosinophil counts done under fasting conditions in thirty patients with Addison's disease failed to reveal a significantly higher level

vation suggests that factors other than adrenal cortical hormone output regulate the basal level of circulating eosinophils. As one of the limitations of the ACTH response it should be noted that both ACTH and Compound F administered at the height of an acute allergic eosinophilia failed to reduce the level of circulating eosinophils to the extent observed in other disorders.

The exact mechanism whereby eosinophils disappear from the circulating blood following adrenal cortical stimulation with ACTH is not known.

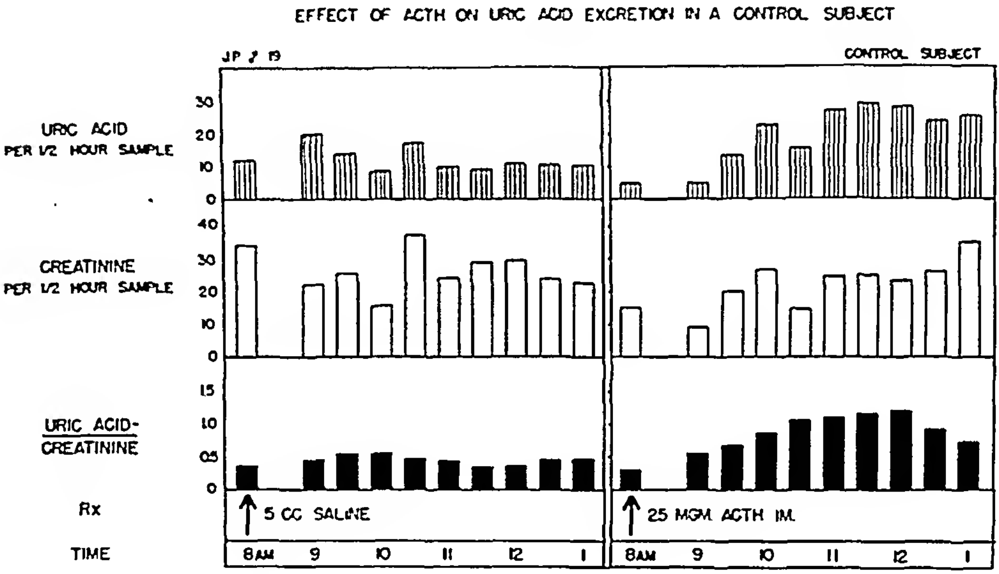

FIG. 5

Neither the spleen nor the thymus appears essential for the phenomenon, as a normal response to ACTH has been observed in man following splenectomy or thymectomy (33).

Metabolic Changes

Uric Acid Excretion: It is known that a variable increase in nitrogen excretion follows the administration of adrenal cortical extract and certain adrenal steroids. Experiments in man indicated that the rise in uric acid excretion following the repeated and prolonged administration of 11 and 11-17-oxysteroids (30) was much more consistent and of relatively greater magnitude than the rather inconstant increase in total nitrogen excretion. With these facts in mind, uric acid excretion was studied as a possible indicator of increased adrenal cortical secretion of carbohydrate-regulating factors.

Following the intramuscular injection of 25 mg. of ACTH in the fasting

state, an increase in uric acid excretion was observed in ten normal subjects and in forty patients with apparently adequate adrenal cortical reserve. The maximum increase in uric acid excretion occurred approximately four hours following the administration of hormone (Fig. 5). This corresponded with the point of maximal eosinophil and lymphocyte depression.

TABLE 5. PERCENTILE RISE IN URINARY URIC ACID-CREATININE RATIO FOLLOWING THE ADMINISTRATION OF 25 MG. OF ACTH

Group	Number of Subjects	6:00 a.m.–8:00 a.m. Ratio	9:00 a.m.–12:00 n. Ratio	Change
Normal Subjects	10			
Mean		0.49 ±0.10	0.93 ±0.15	+91% ±23
Range		(0.35 to 0.68)	(0.67 to 1.15)	(+62 to +130)
Males	6	0.43	0.85	100%
Females	4	0.59	1.06	78%
Non-Addisonians	40			
Mean		0.55 ±0.15	1.01 ±0.23	+89% ±29
Range		(0.27 to 0.92)	(0.59 to 1.62)	(+28 to +172)
Males	15	0.55	0.95	+81%
Females	25	0.56	1.04	+93%
Addisonians	30			
Mean		0.56 ±0.12	0.64 ±0.12	+16% ±13.2
Range		(0.31 to 0.99)	(0.34 to 1.14)	(−14 to +59)
Males	14	0.48	0.59	+21%
Females	16	0.61	0.67	+10%

Creatinine excretion remained constant or decreased as a rule. With this in mind changes in uric acid were computed as differences in the uric acid-creatinine ratio, thereby obviating the necessity for complete urine collections and accurate volume measurements. The uric acid-creatinine ratio in urine specimens collected from 6:00 a.m. to 8:00 a.m. following an overnight fast proved to be remarkably constant from day to day in the same individual. Furthermore, the uric acid-creatinine ratio in a specimen collected from 9:00 a.m. to 12:00 n. under fasting conditions varied little from the early morning 6:00 to 8:00 specimen (Fig. 5). Since little increase in uric acid excretion occurred during the first hour following ACTH administration (Fig. 5), the uric acid-creatinine ratio in the control specimen from 6:00 a.m. to 8:00 a.m. was compared with that of the 9:00 a.m. to 12:00 n. specimen, the injection of ACTH having been given at 8:00 a.m. In patients with marked creatinuria the uric-acid creatinine

ratio tends to be abnormally high but falls within the normal range when total rather than preformed creatinine is used.

In a group of ten normal subjects the increase in uric acid-creatinine ratio following ACTH administration amounted to 91 per cent (plus 62 to plus 130): in forty patients with diseases apparently not involving the adrenal cortex the increase in ratio was 89 per cent (plus 28 to plus 172); whereas in thirty patients with Addison's disease a mean increase of only

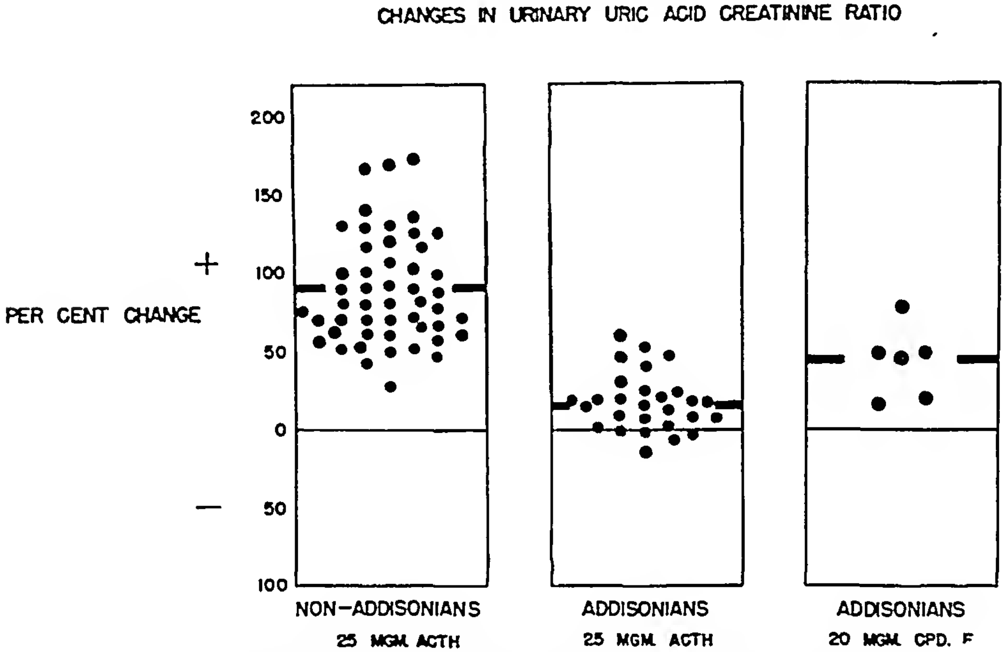

FIG. 6. Each point represents the result obtained on one subject. The heavy horizontal bars mark the mean value for the group.

16 per cent (minus 14 to plus 59) was noted (Table 5, Fig. 6). Alth ugh there was a striking difference in the response of the Addisonian and non-Addisonian groups as a whole to ACTH administration, there was considerable overlapping in individual instances. The initial control uric acid-creatinine ratios (6:00 a.m. to 8:00 a.m.) were remarkably similar for the three groups (Table 5).

A number of patients with Addison's disease who failed to show an increase in uric acid-creatinine ratio following ACTH administration were given 20 mg. of Compound F. Although the ratio increased in all patients, the magnitude of the change was not as great as that observed in normal individuals given 25 mg. of ACTH (Table 6, Fig. 6). It is interesting to note that 20 mg. of Compound F induced a near maximal fall in circulating eosinophils. Since it was impossible, because of lack of material, to give

these patients larger doses of Compound F, it cannot be stated whether the failure to induce a maximal increase in uric acid-creatinine ratio was due to a quantitative or qualitative inadequacy of Compound F.

Two patients given a single injection of a water soluble preparation of Compound A in a dose of 25 and 50 mg. respectively showed no increase in uric acid-creatinine ratio, although in previous studies (30) repeated administration of Compound A over a period of several days had increased the uric acid-creatinine ratio in most instances. The injection of a relatively large dose of water soluble desoxycorticosterone glucoside was fol-

TABLE 6. CHANGES IN URIC ACID-CREATININE RATIO FOLLOWING THE ADMINISTRATION OF VARIOUS WATER SOLUBLE STEROID HORMONES TO PATIENTS WITH ADDISON'S DISEASE

Patient	Sex	Age	Hormone	Dose mg.	Uric Acid-Creatinine Ratio 6:00 a.m.–8:00 a.m.	Uric Acid-Creatinine Ratio 9:00 a.m.–12:00 n.	Change
M.N. H.P.	F F	48 33	17-hydroxy-corticosterone (Compound F)	20 20	0.47 0.43	0.80 0.63	+48% +45%
J.B. E.C.	M M	56 13	11-dehydrocorticosterone hemisuccinate (Compound A)	25 50	0.33 0.65	0.35 0.66	+ 7% + 2%
C.S. J.G.	F F	44 37	Desoxycorticosterone glucoside	30 30	0.50 0.54	0.61 0.68	+22% +27%
E.Z.	F	58	Testosterone diethyl-amino-ethyl-carbonate hydrochloride	30	0.45	0.37	−18%

lowed by a small but definite increase in ratio. The only fall in uric acid-creatinine ratio observed in these experiments occurred in a patient given a water soluble testosterone preparation (Table 6).

The rise of 16 per cent in uric acid-creatinine ratio observed in patients with Addison's disease may be taken as the limit of the ACTH response not mediated through the adrenal cortex or may represent the effect of some remaining active cortical tissue. To test the possibility that the activity of contaminating substances in the ACTH preparation might have contributed to the increase in uric acid-creatinine ratio in subjects with adequate adrenal cortical function, the ratio was followed in three indi-

viduals given pitocin and pitressin in a dosage ten times as large as that contained in 25 mg. of ACTH. Only one of the three subjects showed a definite rise (plus 29 per cent) in uric acid-creatinine ratio. Thus the possibility that contaminating substances in the ACTH preparations might have acted as "alarming agents" cannot be eliminated, although it seems unlikely in view of the small changes observed following the relatively large dosage of posterior pituitary hormone.

The increase in uric acid excretion observed with ACTH must be due to either increased production, increased clearance, or both. In favor of an increased uric acid clearance is the fact that the majority of patients showed no significant rise in serum uric acid level during a period of in-

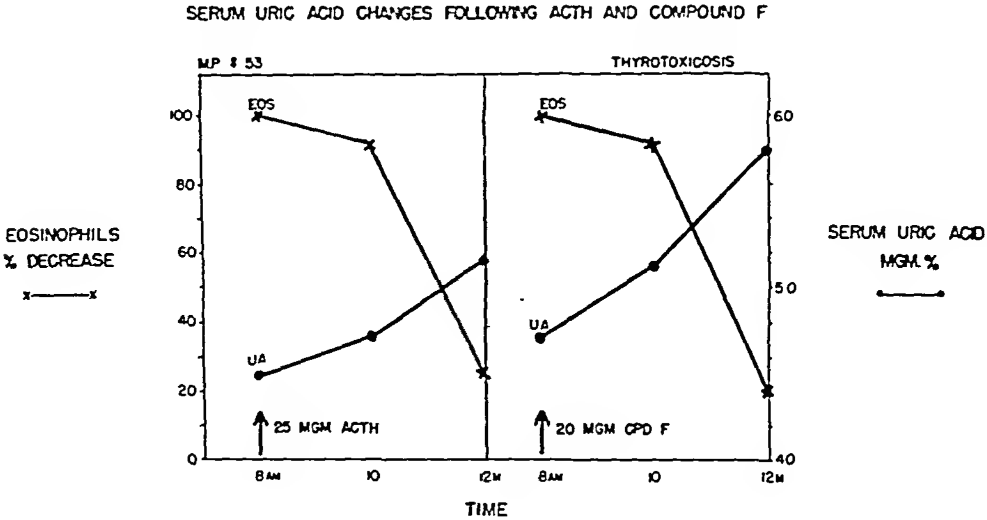

FIG. 7

creased uric acid excretion. Increased formation or mobilization of uric acid following ACTH administration was suggested in a group of patients with mild renal impairment who showed a rise in serum uric acid level with a slight rise in uric acid excretion (patient M.P., Fig. 7). In the same subject similar changes were produced by the administration of 20 mg. of Compound F. From this it appeared that the increased excretion of uric acid which followed ACTH administration was in all probability due to an increase both in formation and clearance.

Increased uric acid excretion as an index of increased adrenal cortical function has certain limitations which should be pointed out. Under conditions of dehydration, hyperuricemia (gout or leukemia), or renal insufficiency, uric acid clearance may already be approaching the maximum. Hence an appreciable increase in uric acid excretion cannot occur in response to ACTH, although one would anticipate a rise in serum uric acid

levels under such circumstances. In patients with severe liver damage the formation of uric acid from its precursors may be impaired, and hence only a limited rise in serum uric acid level and in uric acid excretion should be anticipated in response to ACTH. Finally, depletion of the immediate source of uric acid, thought by some to be lymphoid tissue, or a reduction in the rate of its breakdown as in hypothyroid states, might limit the magnitude of the uric acid response to ACTH.

Electrolyte Excretion: Adrenal cortical stimulation might be expected to induce variable changes in electrolyte excretion, depending upon the relative proportions of salt-retaining and salt-losing adrenal steroids secreted. In these studies electrolyte excretion might also be affected by the posterior pituitary contaminants in various ACTH preparations.

TABLE 7. PERCENTILE CHANGES IN URINARY CONSTITUENTS FOLLOWING THE ADMINISTRATION OF 25 MG. OF ACTH TO SUBJECTS WITH NORMAL ADRENAL FUNCTION AND TO PATIENTS WITH ADDISON'S DISEASE

	Patient	Sex	Age	Blood Eosinophil Responses	Uric Acid Creatinine	Total Nitrogen Creatinine	Potassium Creatinine	Sodium Creatinine	Chloride Creatinine	Inorganic Phosphorus Creatinine
Non-Addisonians	M.G.	F	23	−88%	+95	−22	+240	+100	+ 67	− 43
	S.M.	F	65	−81%	+81	−10	+168	+126	+167	+100
	L.P.	M	33	−52%	+80	− 2	+235	+ 69	+100	− 26
Addisonians	J.S.	M	45	+26%	+26	− 6	+ 83	+ 9	± 0	+ 3
	M.F.	F	44	− 2%	+19	− 6	+ 11	+ 35	+ 44	+ 37
	E.V.*	M	49	−10%	+12	− 7	+ 1	+ 5	± 0	− 1

* *Patient E.V. had a urea clearance of only 35 per cent of normal, a fact which may contribute to the poor urinary response to ACTH.*

In the short-term experiments, subjects with adequate adrenal cortical reserve, as indicated by a fall in circulating eosinophils and a rise in uric acid-creatinine ratio, were observed to have increased potassium, sodium, and chloride-creatinine ratios following ACTH administration (Table 7). Changes in inorganic phosphorus-creatinine ratio were variable; whereas total nitrogen-creatinine ratio tended to fall. The increase in the sodium and chloride-creatinine ratios in the four-hour experiments are interesting in contrast to the over-all marked retention of these electrolytes which occurred with repeated injections of ACTH over periods of from four to six days. Only the changes in potassium and uric acid excretion were consistent with both single and repeated injections of ACTH. In patients with Addison's disease, potassium, sodium, and chloride-creatinine ratio changed relatively little following ACTH, suggesting that the changes in renal excretion of these electrolytes observed in the non-Addisonian group were dependent upon the adrenal cortex.

It is conceivable that the increased sodium, chloride, and potassium-creatinine ratios which occurred in the first four hours following ACTH administration reflect a predominant secretion of 11-17-oxysteroids as opposed to desoxycorticosterone-like factors under which circumstances an increase in potassium excretion would be accompanied by sodium and chloride retention (69).

A TEST FOR ADRENAL CORTICAL RESERVE

The authors' experience with a large group of patients with Addison's disease suggested that the failure to observe a fall in circulating eosinophils or a rise in uric acid-creatinine ratio following the injection of a single dose of ACTH provides a relatively simple clinical test (71) of adrenal cortical reserve with particular reference to the carbohydrate-regulating factors.

TABLE 8. AN EXAMPLE OF A NORMAL RESPONSE TO 25 MG. OF ACTH (ACTH TEST)

Patient R.P.	Normal Male	Age 21
Circulating Eosinophils	8:00 a.m.	188 per cu. mm.
	12:00 n.	46 per cu. mm.
Percentile Fall	76%	
Urinary Uric Acid	6:00 a.m.–8:00 a.m.	9:00 a.m.–12:00 n.
Mg. per cent	72	156
Creatinine		
Mg. per cent	185	234
Uric Acid-Creatinine Ratio	0.39	0.67
Percentile Rise	72%	

Procedure

No food is permitted after 8:00 p.m. On the following day water is allowed ad libitum, and in addition 200 cc. of water is given at 6:00 a.m., 8:00 a.m., and 10:00 a.m. A control urine specimen is collected from 6:00 a.m. to 8:00 a.m., and an eosinophil count is done at 8:00 a.m. Immediately thereafter 25 mg. of ACTH is injected intramuscularly. Urine is collected from 9:00 a.m. to 12:00 n., and the eosinophil count is repeated at 12:00 n. The percentile decrease in circulating eosinophils is calculated, the two urine specimens are analyzed for uric acid and creatinine, and the uric acid-creatinine ratio is computed. An example is presented in Table 8.

The technique for the direct eosinophil count and the directions for the preparations of the ACTH for injection are to be found on pages 18 and 19.

Interpretation

Normal adrenal cortical reserve is indicated by a fall in eosinophils and a rise in the uric acid-creatinine ratio exceeding 50 per cent. Mild adrenal insufficiency is indicated by a normal fall in eosinophils with only a small rise in uric acid-creatinine ratio (20 to 50 per cent). Addison's disease is characterized by a decrease in eosinophils of less than 20 per cent and an increase in the uric acid-creatinine ratio not exceeding 50 per cent.

TABLE 9. ACTH RESPONSE IN PATIENTS WITH ANTERIOR PITUITARY INSUFFICIENCY

Patient	Sex	Age	Diagnosis	Degree of Anterior Pituitary Insufficiency	Change in Circulating Eosinophils	Change in Uric Acid-Creatinine Ratio
I.L.	F	42	Simmonds' Disease	Severe	+24%	+ 1%
R.G.	F	33	Simmonds' Disease	Severe	− 4%	+18%
A.P.	F	61	Simmonds' Disease	Moderate	−17%	+22%
M.A.	M	58	Chromophobe adenoma	Severe	−15%	+32%
M.F.	M	60	Chromophobe adenoma	Mild	−27%	+49%
M.R.	M	46	Chromophobe adenoma	Mild	−45%	+90%

The majority of patients with advanced anterior pituitary insufficiency show little if any response to ACTH; whereas cases with milder insufficiency may give some evidence of adrenal cortical stimulation following ACTH administration. Table 9 illustrates the authors' limited experience with this group. Patient R.G., a case of Simmonds' disease of long standing with a negative ACTH test, failed to show any response even after three days of continued ACTH administration (40 mg. per day). On the other hand, patient M.R., who showed a somewhat inadequate response to a test dose of ACTH, revealed full adrenal cortical activity after six days of administration of this material (40 mg. per day). It would appear that the adrenal cortex must have undergone considerable functional if not histologic atrophy in most instances, leaving only an occasional patient with

sufficient potential reserve to respond adequately to ACTH administration over a relatively short period of time. A low basal metabolic rate appears to be another factor in the reduction of the adrenal cortical response.

An overactive adrenal cortex with little functional reserve (alarm reaction (59)) is suggested by a normal fall in eosinophils (50 per cent or more) in the presence of a high fasting uric acid-creatinine ratio (0.8 or greater) with only a small increase following ACTH administration. The limitations to the interpretation of these changes are discussed on pages thirty-one and thirty-two.

EFFECT OF REPEATED INJECTIONS OF ACTH (FOUR TO SIX DAYS)

Plan of Study

One normal male subject, R.P., and two patients, M.R. and J.W., were selected for these experiments. Patient M.R., a forty-six-year old male,

TABLE 10. DAILY DIETARY INTAKE ON CONSTANT DIET*

Subject	Carbo-hydrate Gm./day	Protein Gm./day	Fat Gm./day	Total Calories cal./day	Fluid cc./day	Na m.Eq.	Cl m.Eq.	N Gm.
R.P.	264	74	99	2,290	2,500	113	135	11.9
J.W.	185	59	63	1,560	2,500	65	82	9.5
M.R.	312	38	35	1,720	2,500	74	94	6.0
					1,600			
					2,000			

* The values for sodium, chloride, nitrogen and protein were obtained by the direct analysis of food aliquots.

had received X-ray treatment one year previously for a pituitary chromophobe adenoma; at the time of the study he showed classical symptoms of early pituitary insufficiency. Patient J.W., a fifteen-year old female, had had amenorrhea, weight loss, anorexia, and asthenia for several months prior to admission. The provisional diagnosis was anorexia nervosa. These two patients were selected with the hope that certain responses to ACTH might be exaggerated, and hence it might be possible to detect changes which would not be apparent or significant in the normal. Patient M.F., a forty-nine-year old housewife with known Addison's disease for six years, served as a control.

All subjects were placed on a constant dietary regimen (Table 10). After a control period in which a steady state had been reached, as estimated from the constancy of urinary chloride and nitrogen excretion, intramuscular injections of ACTH were begun. The hormone was given in divided

doses of 10 mg. per dose every six hours, a total dose equivalent to 40 mg. of the Armour standard preparation per day over a period of from four to six days.

Evidence of increased secretion of adrenal androgens was obtained by the determination of urinary 17-ketosteroid excretion,[9] of 11 and 11-17-oxysteroids by following the excretion of 11-oxysteroids, uric acid, total nitrogen, alterations in glucose metabolism, and the change in circulating eosinophils and lymphocytes, and of desoxycorticosterone-like steroids by following changes in body weight, urine volume, and the excretion of sodium and chloride (74).

General Effects

The repeated administration of ACTH in doses of 10 mg. every six hours over a period of from four to six days was well tolerated by both the normal subject and the patients. In no instance did a significant elevation in systolic or diastolic blood pressure occur. There was evidence of considerable fluid retention in all except the patient with Addison's disease.

Changes in Adrenal Androgenic Steroid Secretion

Urinary Excretion of 17-Ketosteroids: The two patients, M.R. and J.W., and the normal male subject, R.P., showed a significant increase in 17-ketosteroid excretion during the administration of ACTH (Fig. 8). In patient M.R. the increase amounted to approximately 500 per cent, in patient J.W., 300 per cent, and in the normal subject, 100 per cent. An increased excretion of 17-ketosteroids was noted as early as the second day of treatment (M.R.) and was sustained throughout the period of ACTH administration. The values fell rapidly to the pretreatment level within three days following withdrawal of ACTH administration (Fig. 8). Although the excretion of 17-ketosteroids increased greatly during the four to six days of ACTH administration, it is of interest to note that in no instance did the maximum value exceed high normal for that individual.

Further evidence of the secretion of an increased quantity of androgenic steroids occurred in patient M.R. who noted that his beard grew much heavier and continued to do so for some time after discontinuing the ACTH and that it was necessary for him to shave daily instead of twice weekly as had formerly been necessary. He also noticed nocturnal erections which had not been present for at least one year previously.

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[9] It is common practice to assume that an increase in steroid excretion necessarily implies increased hormonal production. In the absence of blood determinations the limitation of this assumption is appreciated.

Patient M.F., an Addisonian treated with ACTH for four days, failed to show any increase in 17-ketosteroid excretion (Fig. 19).

Changes in 11 and 11-17-Oxysteroid Secretion

11-Oxysteroid Excretion: A striking rise in 11-oxysteroid excretion was observed in all three individuals (Fig. 8). It should be noted that the initial

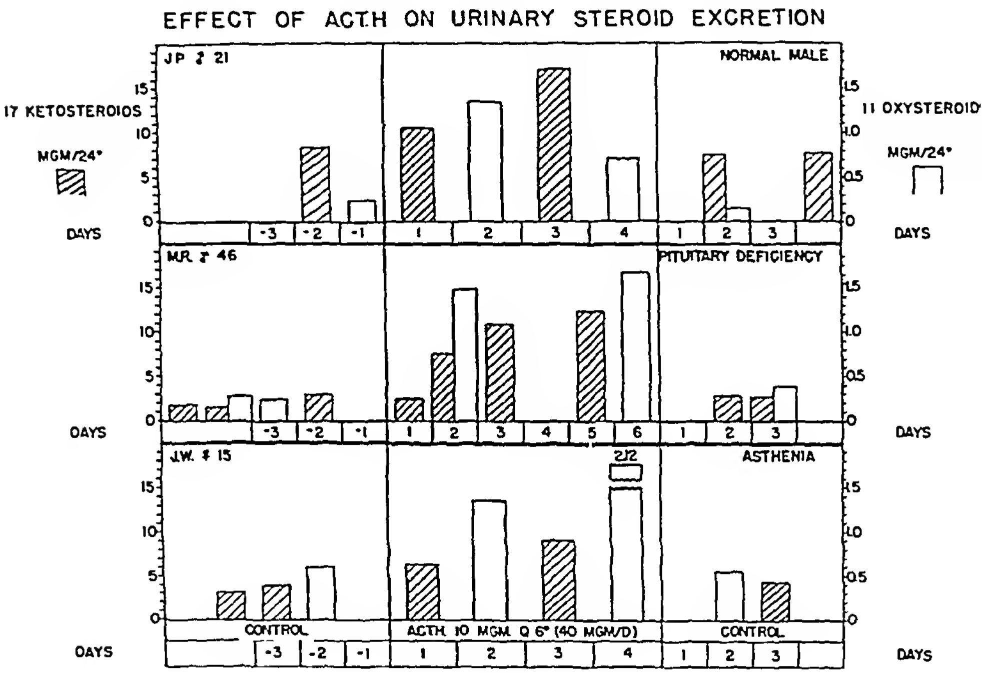

FIG. 8

values during the control period indicated a normal level in patient M.R. and normal subject R.P.; whereas the initial value in patient J.W. was higher than normal. The highest value attained during ACTH administration in all three individuals was of approximately the same order of magnitude, i.e. 1.34 to 2.42 mg. per twenty-four hours, representing percentile increases of 500 per cent for patient M.R., 300 per cent for patient J.W., and 500 per cent for normal subject R.P. Although the excretion of both 17-ketosteroids and 11-oxysteroids was increased greatly by ACTH administration, there was this difference between the two responses: Whereas the excretion of 11-oxysteroids attained abnormally high levels, such as have been noted in Cushing's syndrome (65), the 17-ketosteroid excretion never exceeded high normal levels. Patient M.F. with Addison's disease

failed to show any increase in 11-oxysteroid excretion[10] following four days of ACTH administration (Fig. 19).

Uric Acid Excretion: All three individuals, M.R., J.W., and R.P., showed an increase in uric acid excretion during ACTH administration (Figs. 9, 10, 11) with a maximum rise during the first forty-eight hours. The per-

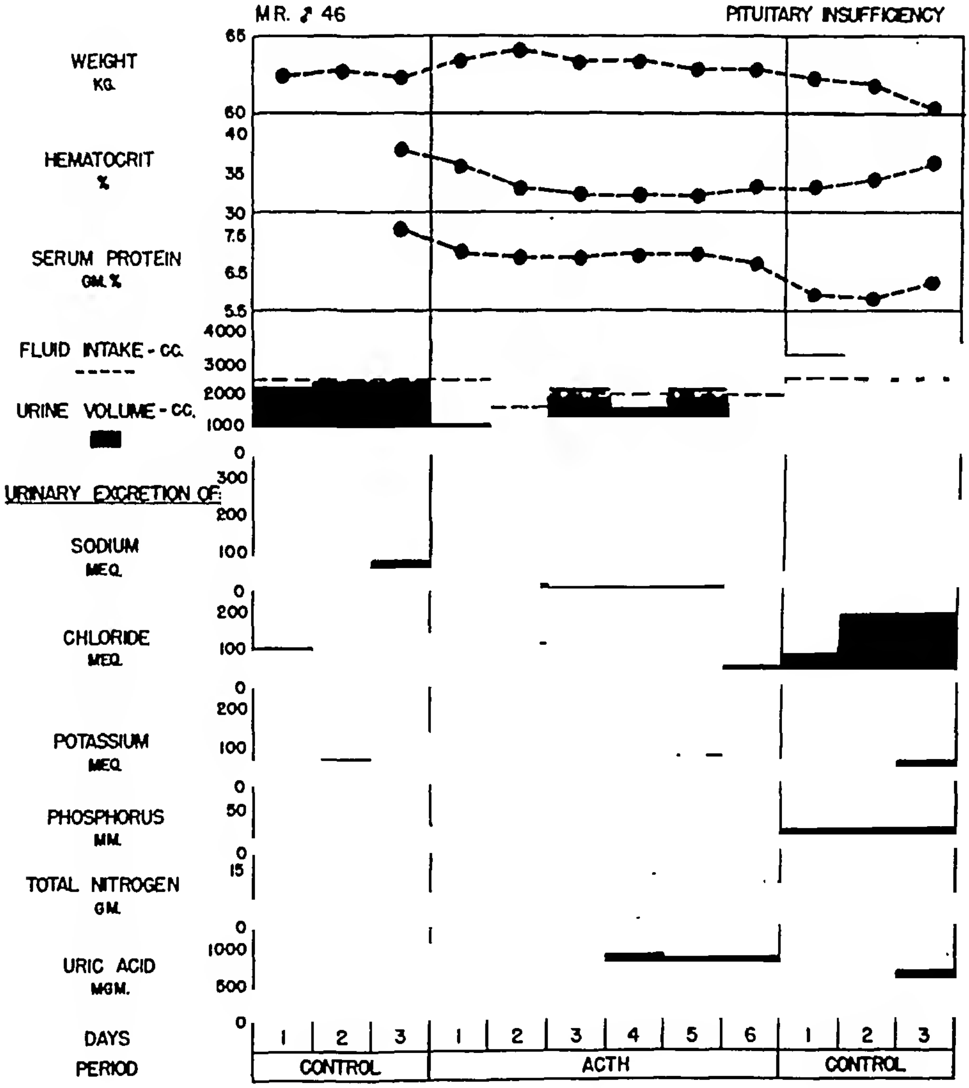

FIG. 9. Summary of some of the metabolic changes following ACTH administration.

centile increase for the entire period of treatment amounted to 92 per cent for patient M.R., 95 per cent for patient J.W., and 20 per cent for normal subject R.P. The increased uric acid excretion was accompanied in all three subjects by a decrease in serum uric acid level greater than could be ex-

10 The authors are indebted to Dr. Nathan B. Talbot for carrying out the 11-oxysteroid determinations reported on patients J.W. and M.F. and on normal subject R.P.

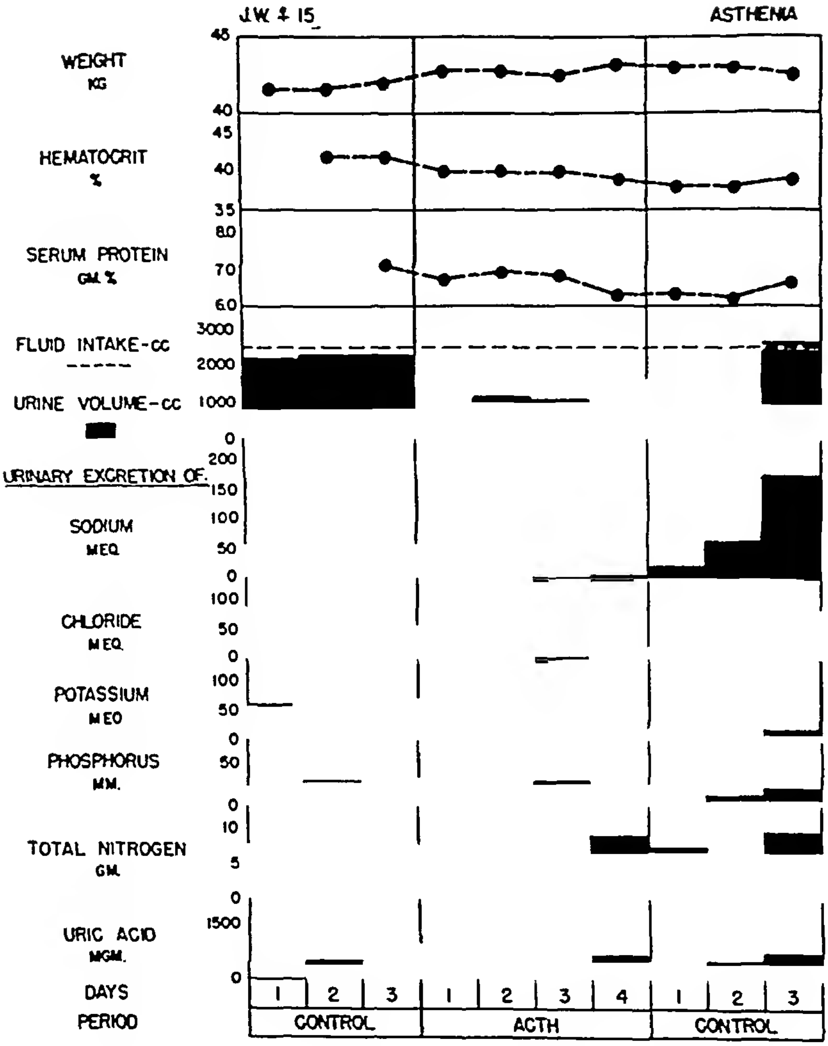

FIG. 10. Summary of some of the metabolic changes following ACTH administration.

plained by the associated hemodilution (Table 11). The fall in serum uric acid level suggests increased uric acid clearance; the magnitude of the increase in uric acid excretion observed during this same period suggests a rise in uric acid production (Fig. 12).

In contrast to the changes in uric acid excretion, no increase in preformed creatinine was observed in any of the three subjects, nor did an appreciable change occur in creatine in the one case in which it was followed (patient J.W.).

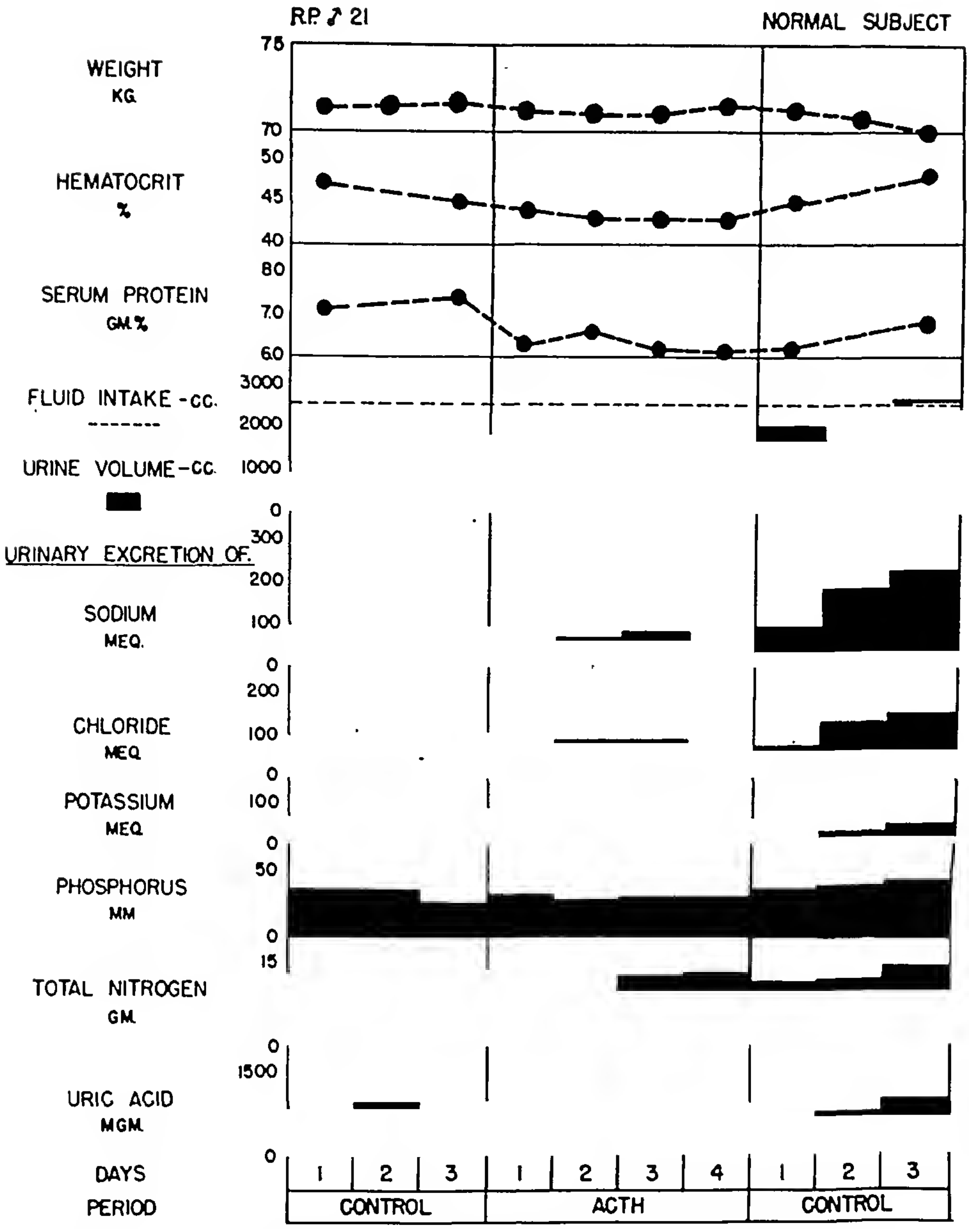

FIG. 11. Summary of some of the metabolic changes following ACTH administration.

Patient M.F. with Addison's disease failed to show a significant increase in uric acid excretion (Fig. 19) or a fall in serum uric acid level during a four-day period of ACTH administration.

Nitrogen Balance: In patient M.R. nitrogen balance changed from minus 2.5 Gm. daily during the control period to minus 5.9 Gm. daily during the six days of ACTH therapy (Table 12). The negative nitrogen balance was accounted for entirely by increased urinary excretion. Normal subject R.P. was in negative nitrogen balance during the preliminary control period (minus 2.7 Gm. daily). During ACTH administration the negative nitrogen

balance decreased to a value of minus 0.5 Gm. daily, returning toward the original value following withdrawal of hormone. In patient J.W. it was possible to follow only urinary nitrogen excretion which decreased slightly from a level of 7.9 Gm. daily to 7.6 Gm. daily during hormone therapy and returned to the control level following withdrawal of ACTH. Patient M.F.

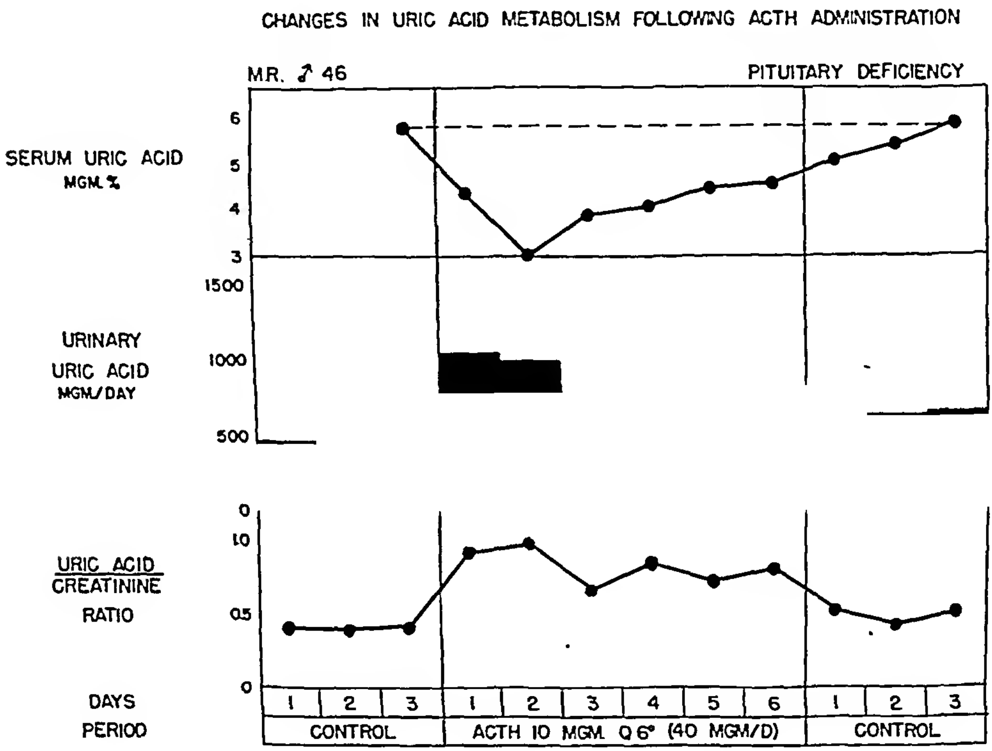

Fig. 12

with Addison's disease showed no significant change in urinary nitrogen excretion during ACTH administration.

Serum Protein: There was a fall in serum protein level in all three individuals during ACTH therapy (Fig. 13). The maximum decrease was 1.3 Gm. per cent in normal subject R.P., 0.8 Gm. per cent in patient J.W., and 1.8 Gm. per cent in patient M.R. A moderate degree of hemodilution accompanied the fall in total serum protein but was insufficient to explain the total decrease in serum protein level. The greatest fall in protein occurred in patient M.R. who was given ACTH for six days and who showed the greatest increase in urinary nitrogen excretion (Fig. 9). It appeared that the second significant drop in serum protein level in this patient occurred

TABLE 11. CHANGES IN CIRCULATING LYMPHOCYTES, SERUM URIC ACID AND URINARY URIC ACID EXCRETION FOLLOWING THE ADMINISTRATION OF ACTH

		J.W.				M.R.				R.P.			
Periods	Days	Htc. %	Lym. no./cu. mm.	Serum Uric Acid mg. %	Urinary Uric Acid mg./day	Htc. %	Lym. no./cu. mm.	Serum Uric Acid mg. %	Urinary Uric Acid mg./day	Htc. %	Lym. no./cu. mm.	Serum Uric Acid mg. %	Urinary Uric Acid mg./day
Control	1								463	47	2,300	6.7	839
	2	42			518		4,000		454			6.3	910
	3	42	2,520	4.5	420	38	5,550	5.8	492	45	3,400	6.5	850
ACTH 40 mg. per day	1	40	1,400	4.2	1,023	36	1,680	4.4	1,064	44	2,390	5.3	1,000
	2	40	2,370	3.3	1,532	33	1,320	3.0	998	43	2,570	5.1	1,126
	3	40	2,730	3.5	476	32	1,460	3.9	787	43	2,930	5.1	1,110
	4	39	3,430	3.1	622	32	2,050	4.1	898	43	3,310	4.4	910
	5					32	2,010	4.5	836				
	6					33	2,300	4.6	835				
Control	1	38	4,000	4.9	416	33	2,800	5.1	623	45	5,680	5.4	637
	2	38	3,170	4.2	512	34	2,300	5.4	535	48		5.9	748
	3	39	3,080		782	36	2,130	5.9	656	46	3,850	6.1	1,002

TABLE 12. CHANGES IN NITROGEN BALANCE DURING ACTH ADMINISTRATION

	Normal Subject R.P.			Patient M.R.		Patient J.W.		
	Control	ACTH	Control	Control	ACTH	Control	ACTH	Control
Nitrogen Excretion Gm. per day								
Urinary	13.4	11.6	12.3	7.6	10.9	7.9	7.6	7.9
Fecal	1.2	0.8	1.3	0.7	0.8	—	—	—
Total	14.6	12.4	13.6	8.3	11.7			
Nitrogen Intake Gm. per day	11.9	11.9	11.9	5.8	5.8	9.5	9.5	9.5
Balance	−2.7	−0.5	−1.7	−2.5	−5.9	(+1.6)	(+1.9)	(+1.6)

after a urinary loss of approximately 20 Gm. of nitrogen, equivalent to 125 Gm. of body protein (Fig. 14). The changes in serum protein pattern (electrophoretic analysis)[11] in patient M.R. and normal subject R.P. are

[11] The authors are indebted to Dr. S. Howard Armstrong, Jr., for the electrophoretic patterns and their analysis (46).

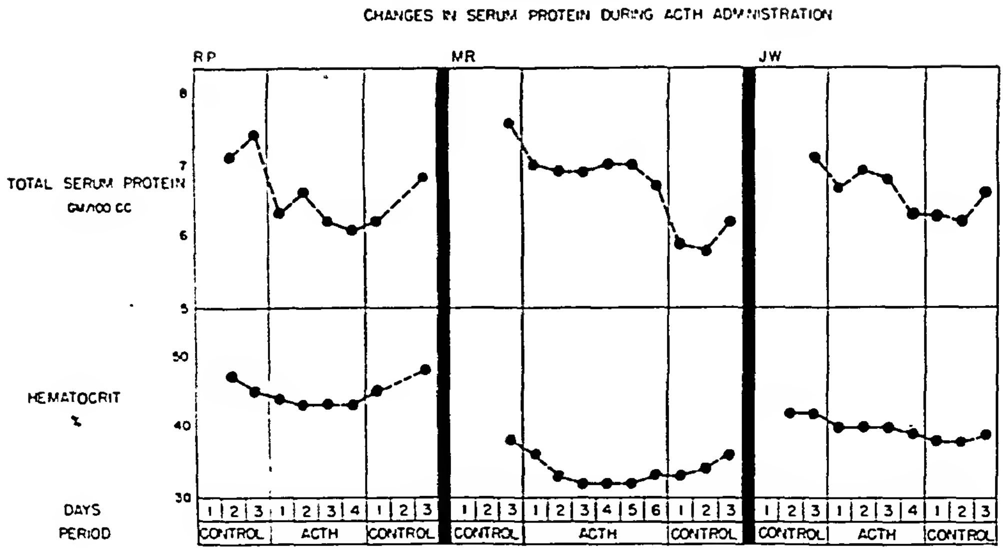

FIG. 13

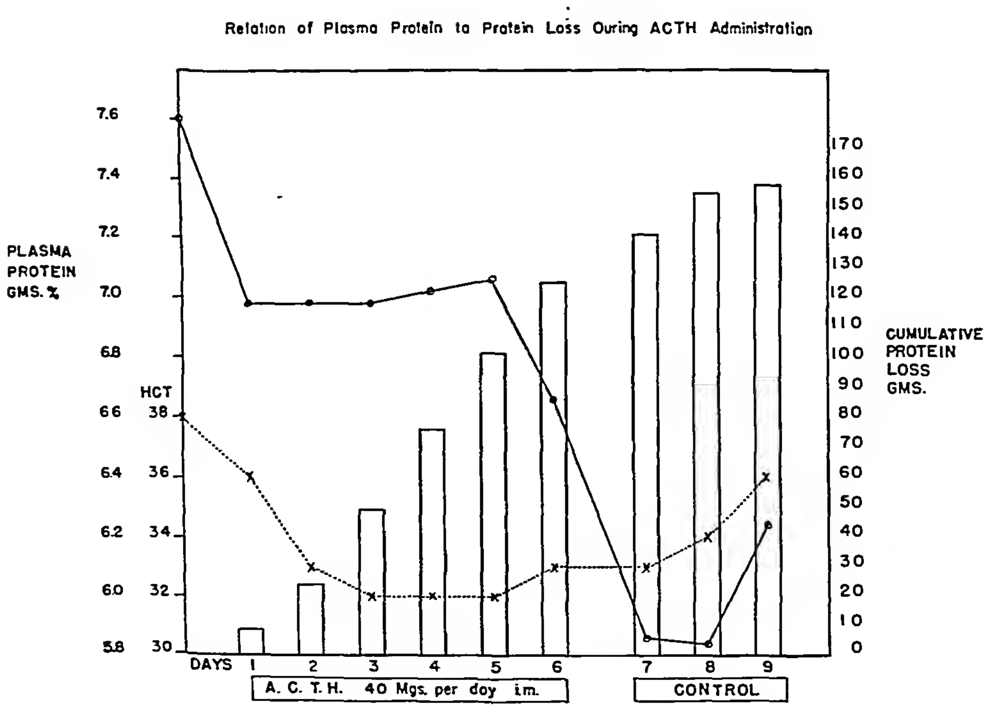

FIG. 14. The cumulative nitrogen loss was calculated from excess nitrogen excretion based on the original control level.

summarized in Table 13. In patient M.R. given ACTH for six days, there was a significant rise in albumin with a reciprocal fall in alpha-2 globulin, changes opposite to those found in patients with Addison's disease. The small rise in gamma globulin is hardly significant. In normal subject R.P. there was no significant alteration in the electrophoretic pattern following four days of ACTH administration.

TABLE 13. CHANGES IN SERUM PROTEIN ELECTROPHORETIC PATTERN DURING ACTH ADMINISTRATION

Patient M.R.

Rx	Total Protein Gm. %	Serum Component per cent				
		Albumin	Globulin			
			α_1	α_2	β	γ
Control	7.6	50	3	25	6	11
After 3 days of ACTH Rx	7.0	57	4	14	8	14
After 5 days of ACTH Rx	7.0	57	4	15	6	14
3 days after withdrawal of ACTH	6.2	52	4	21	9	10

Normal Subject R.P.

Rx	Total Protein Gm. %	Serum Component per cent				
		Albumin	Globulin			
			α_1	α_2	β	γ
Control	7.1	60	4	8	12	14
After 4 days of ACTH Rx	6.2	59	5	9	13	14

It is possible that the variable changes in nitrogen balance in these three individuals reflected differences in the over-all effect of increased androgenic versus 11-oxysteroid secretion, the former predisposing to nitrogen retention and the latter to nitrogen loss. The marked differences in nitrogen balance are to be contrasted with the consistent and uniform increase in uric acid excretion which occurred in all three subjects during ACTH administration (Figs. 9, 10, 11).

Inorganic Phosphorus: Urinary excretion of inorganic phosphorus was greatly increased in patient M.R. during the first four days of ACTH administration (Fig. 9). In patient J.W. there was an increased phosphorus excretion (plus 7.2 m.mol.) during the first day of ACTH treatment (Fig. 10). In contrast, normal subject R.P. showed a slightly diminished phosphorus excretion during ACTH administration with a rise following the withdrawal of ACTH (Fig. 11). The initial phosphorus diuresis observed in patients M.R. and J.W. preceded the maximum increase in nitrogen excre-

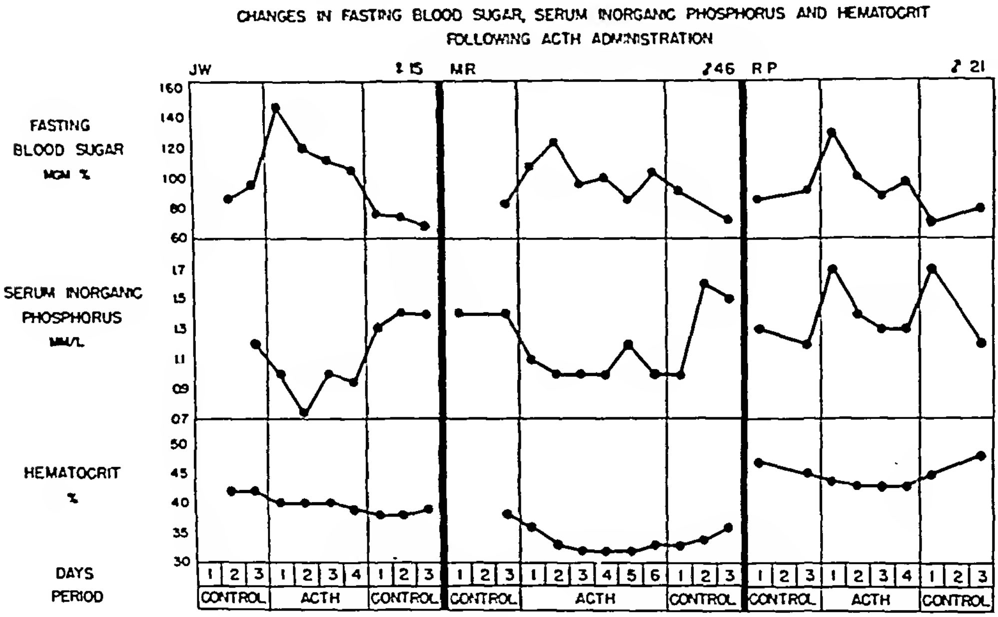

FIG. 15

tion in M.R. and was unaccompanied by any increase in total nitrogen in patient J.W.

The variable change in urinary phosphorus excretion in these three individuals paralleled the changes in serum inorganic phosphorus. There was a fall of 0.4 m.mol. in patient M.R. and a fall of 0.2 m.mol. in patient J.W.; whereas normal subject R.P. showed an over-all rise of 0.2 m.mol. (Fig. 15). A reciprocal relation of the changes in serum inorganic phosphorus to those in fasting blood sugar was observed in patients M.R. and J.W. but not in the normal male R.P.

No change in the excretion of inorganic phosphorus or the level of serum inorganic phosphorus was observed in patient M.F. with Addison's disease.

Urinary Calcium: During the control period and during the six days of ACTH administration in patient M.R. no appreciable change in the excre-

tion of calcium was observed, the mean values being 90 and 93 mg. daily respectively for these periods. During the three days following the withdrawal of ACTH, however, a large increase in calcium excretion was noted concurrently with a marked diuresis (134 mg. average daily excretion).

Urinary Potassium: An increase in urinary potassium excretion occurred in all three individuals during the first twenty-four to forty-eight hours on

RX	DAILY URINARY EXCRETION OF:		
	NITROGEN GM.	PHOSPHORUS MM.	POTASSIUM M.EQ.
ACTH	10.9	26	65
O	7.6	19	27
DIFFERENCE	- 3.3	- 7	- 38

THEORETICAL RATIO FOR PROTOPLASM

$N/P = 15$ $K/N = 2.7$

OBSERVED RATIO

$N/P = \frac{3.3}{7 \times 0.031} = 15.2$ $K/N = \frac{38}{3.3} = 11.5$

CONCLUSION

P EXCRETION MAINLY DERIVED FROM PROTOPLASMIC BREAK-DOWN

K EXCRETION 4.25 TIMES GREATER THAN IF IT WERE DERIVED FROM PROTOPLASM

THE RELATION OF NITROGEN LOSS TO INORGANIC PHOSPHORUS AND POTASSIUM LOSS IN PATIENT M.R. GIVEN ACTH

N.B. CHANGES IN FECAL EXCRETION SHOWN TO BE NEGLIGIBLE IN THE CASE OF NITROGEN ARE "ASSUMED" TO BE SUCH FOR PHOSPHORUS AND POTASSIUM AS WELL

FIG. 16

ACTH. The initial maximum increase amounted to 18 m.Eq. per day in patient M.R., 9 m.Eq. per day in patient J.W., and 20 m.Eq. per day in normal subject R.P. (Figs. 9, 10, 11). The increase in potassium excretion was unaccompanied by an increase in the total nitrogen excretion in two cases (J.W. and R.P.).

In an attempt to determine to what extent the increase in inorganic phosphorus and potassium excretion reflected the breakdown of body pro-

tein in patient M.R., appropriate calculations were made (Fig. 16 (54)). These suggested that, whereas the over-all increase in inorganic phosphorus excretion correlated closely with the breakdown of body protein, potassium excretion exceeded by five times the anticipated ratio. In this regard it should be noted that the increased potassium excretion occurred in conjunction with marked sodium and chloride retention analogous to changes observed following desoxycorticosterone administration (72).

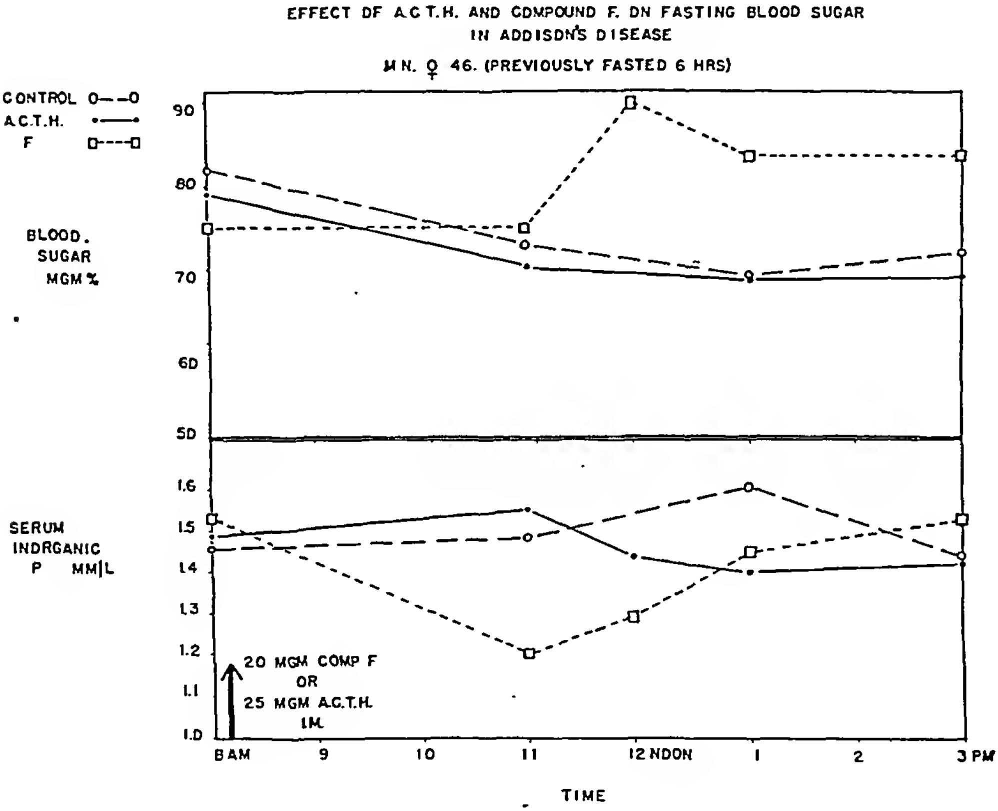

Fig. 17

Carbohydrate Metabolism: Fasting blood sugar levels rose slightly in all three individuals during a four to six day period on ACTH (Fig. 15). The mean increase in fasting blood sugar level during ACTH administration amounted to 26 mg. per cent in subject R.P., 31 mg. per cent in patient J.W., and 15 mg. per cent in patient M.R. Glycosuria was not observed in any instance. Following the withdrawal of ACTH, the fasting blood sugar level fell promptly in all three individuals to values somewhat below the pretreatment control. In all instances blood for fasting blood sugar determinations was drawn five to six hours after the administration of 10 mg. of

ACTH in order to minimize the possibility of an immediate hyperglycemic effect of ACTH or contaminants such as pituitrin. Patient M.F. with Addison's disease showed no increase in fasting blood sugar level during a four-day period of ACTH administration.

Crystalline Compound F was administered to a patient with Addison's disease (M.N.) during a prolonged period of starvation in order to determine whether such an individual who had failed to respond to ACTH was capable of responding to an 11-17-oxysteroid preparation (Fig. 17). Under control conditions there was a gradual fall in blood sugar level throughout

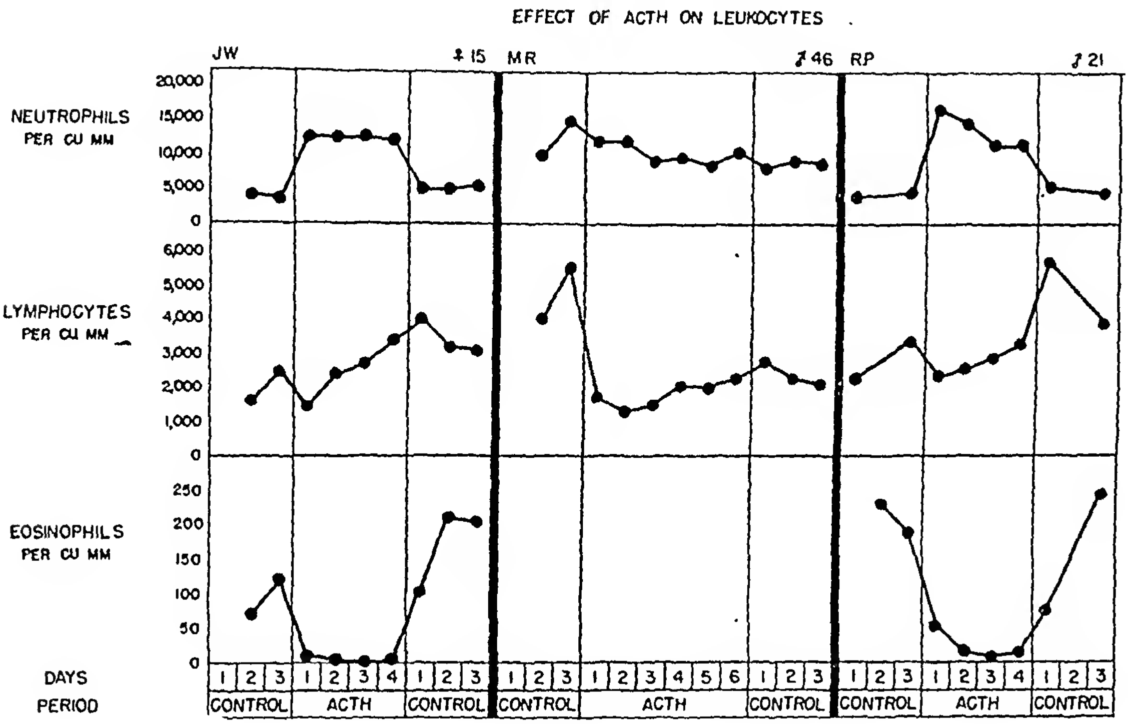

FIG. 18

a thirteen-hour period. A similar fall in blood sugar level was observed with ACTH treatment. With Compound F therapy not only was the progressive fall in blood sugar prevented, but the level of blood sugar at the termination of the fast exceeded the preinjection level.

Changes in Liver Glycogen: Liver biopsies were carried out in an attempt to determine what changes occurred in liver glycogen content under the influence of ACTH. Patient M.R., who was known to respond to ACTH, was maintained on a constant diet (C 250, P 90, F 60) under control conditions for three days prior to operation. Identical preoperative management was carried out in two instances, the first time under control conditions and on a second occasion following the administration of ACTH,

10 mg. every six hours for forty-two hours. In the control study glycogen determinations (31) done on three separate specimens of liver removed eight hours postprandially revealed a mean value of 6.5 Gm. per cent (wet weight); whereas three specimens removed under identical conditions following ACTH treatment were found to contain 8.7 Gm. per cent of gly-

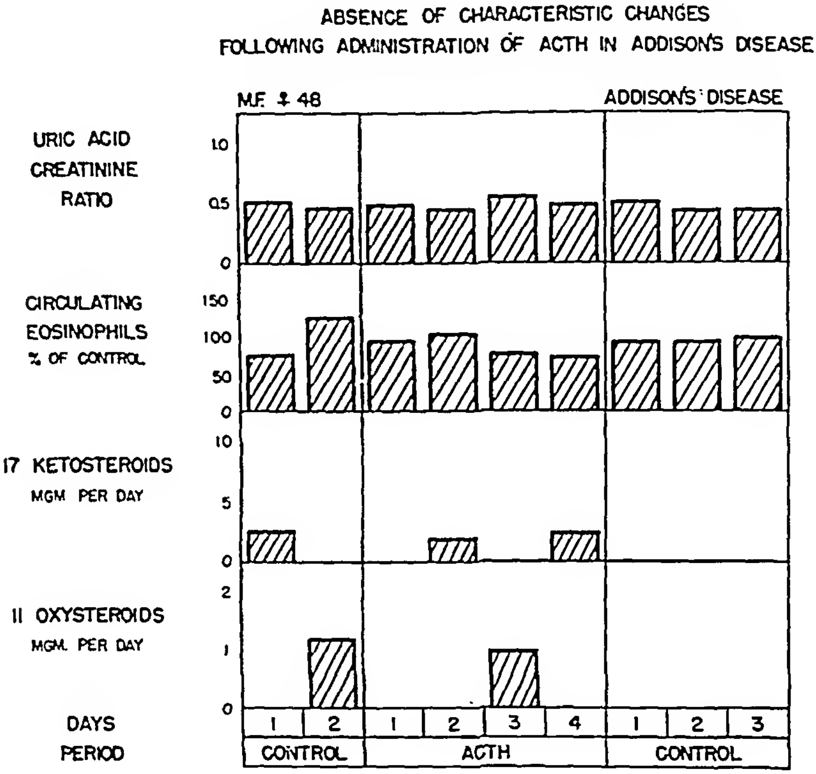

Fig. 19

cogen. The fasting blood sugar values corresponding to the two liver glycogen biopsies were 74 and 104 mg. per cent respectively.

Hematologic Changes: Following ACTH in the prolonged experiments the hematologic changes paralleled those observed following a single injection of 25 mg. of ACTH and are summarized in Fig. 18. A striking fall in circulating eosinophils was observed in the two individuals in whom they were studied by direct count. Following the withdrawal of ACTH, there was a prompt return to normal. In patient M.R. a prolonged and sustained fall in circulating lymphocytes was observed (Fig. 18); whereas patient J.W. and normal subject R.P. showed only a transitory depression with a gradual rise above preinjection levels. All three individuals showed a marked and sustained rise in neutrophils.

In patient M.F. with Addison's disease none of these effects was observed except a slight and delayed fall in circulating eosinophils of 24 per cent with a prompt return to control levels after discontinuing the ACTH (Fig. 19). The absence of the typical eosinophil response in this patient correlated well with the absence of other changes and should be contrasted with the positive changes observed in patient J.W. and summarized in Fig. 20.

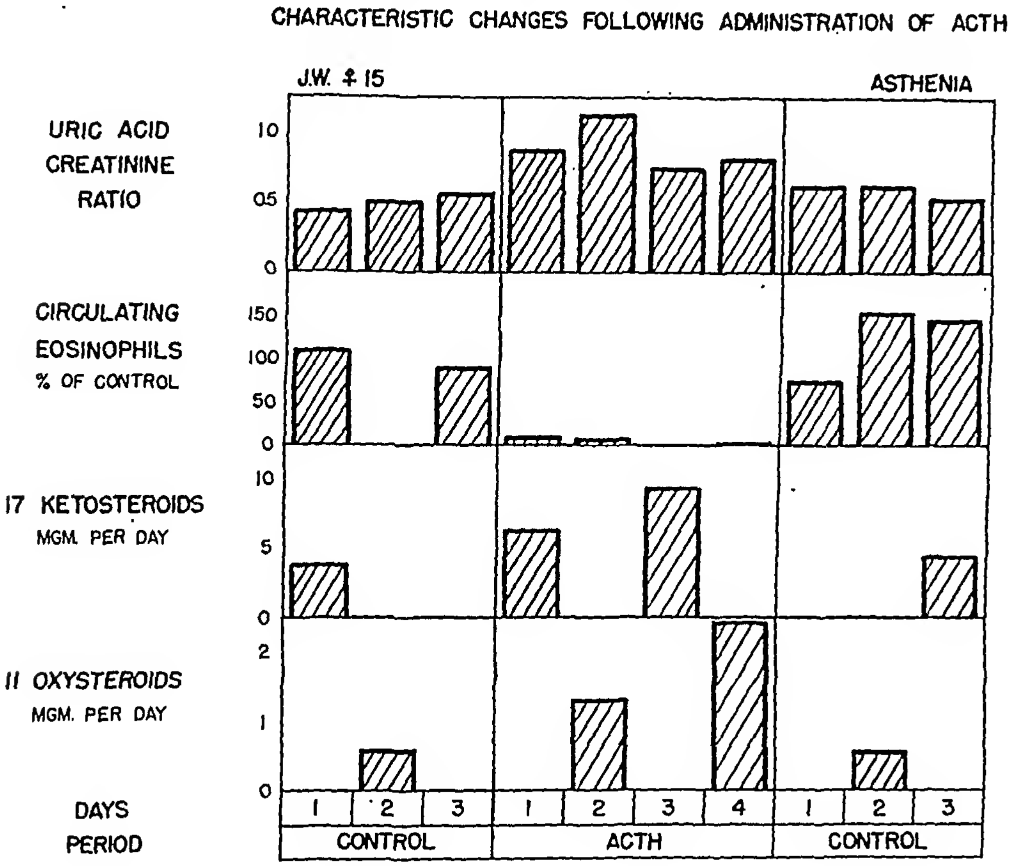

FIG. 20

Antibody Titer: No definite rise in circulating antibodies could be demonstrated following ACTH administration in a small group of human subjects.

Following the administration of a single dose of 25 mg. of ACTH to two patients with latent syphilis no increase in the titers of a specific flocculation test (Hinton) was observed. No change in the titer of typhoid antibodies was noted in a patient given 25 mg. of ACTH who had been immunized one year previously. No response was obtained following ACTH (single dose of 25 mg.) in the titer of mumps antibodies as determined by the complement fixation test in a patient who had had mumps several

years previously. An attempt was made to immunize patient M.R. with a pure pneumococcus Type I polysaccharide, and his precipitin titers were followed during and after the six-day treatment period with ACTH (40 mg. daily). Although there was a small rise in titer which persisted for approximately one week following the administration of ACTH, it cannot be considered significant. It should be noted that in this patient there was a sustained depression of the circulating lymphocytes and a borderline increase in gamma globulins.[12]

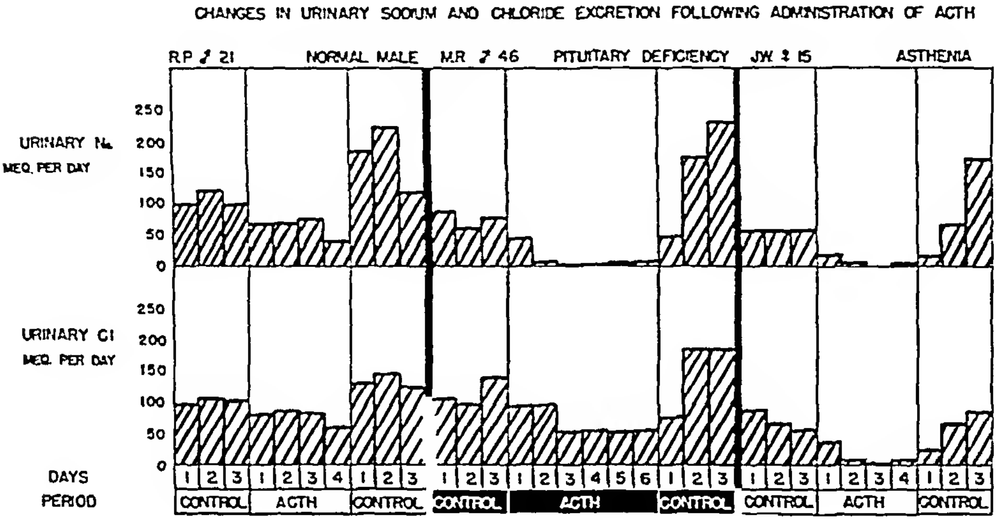

FIG. 21

Changes Suggesting Alteration in the Secretion of Desoxycorticosterone-Like Steroids

During ACTH administration there was a small increase in body weight and a striking decrease in sodium excretion in all three individuals. The latter amounted to 80 per cent in patients M.R. (Fig. 9) and J.W. (Fig.10) and to more than 40 per cent in the normal male R.P. (Fig. 11). The changes in urinary chloride excretion paralleled those of sodium but were considerably less in magnitude (Fig. 21). Following withdrawal of ACTH there was a marked sodium and chloride diuresis in which the increased excretion closely approximated the total quantity of these electrolytes retained during the period of ACTH administration. It is probable that the changes in sodium and chloride excretion in the urine actually reflect bal-

[12] The authors are indebted to Dr. Joseph W. Ferrebee for carrying out some of the immunological studies reported.

TABLE 14. CHANGES IN HEMATOCRIT (HTC.) SERUM DIOXIDE COMBINING POWER, (CO_2), CHLORIDE (CL) AND SODIUM (NA), DURING ACTH ADMINISTRATION

Period	Subject R.P.				Patient J.W.				Patient M.R.			
	Hct. %	CO_2 m.M./L	Cl m.Eq./L	Na m.Eq./L	Hct. %	CO_2 m.M./L	Cl m.Eq./L	Na m.Eq./L	Hct. %	CO_2 m.M./L	Cl m.Eq./L	Na m.Eq./L
Control	45	27	104	144	42	24.4	103	138	38	24	109	142
ACTH (40 mg./day)	43	28	107	149	40	26.2	101	139	33	28	103	137
Control	45	29	107	145	38	27.2	106	140	34	32	103	147

The figures represent the average of several daily determinations during the experimental period.

ance changes, since in two patients a study of fecal chloride excretion showed only insignificant changes during the period of ACTH administration. The prompt reduction in sodium excretion with ACTH treatment, the magnitude of the change, and the "rebound" phenomena during the withdrawal period are analogous to changes observed with synthetic desoxycorticosterone treatment (72). This analogy is further supported by the transitory increase in urinary potassium excretion which occurred in all three individuals in association with sodium and chloride retention which greatly exceeded the relative quantities of nitrogen and inorganic phosphorus excreted during the same period.

Changes in the serum level of sodium, chloride, and carbon dioxide combining power were followed throughout the experimental periods (Table 14). There was a tendency for serum carbon dioxide combining capacity to be increased at the expense of chloride, a change similar to that observed in certain patients with Cushing's syndrome (14).

It is conceivable that increased adrenal androgen secretion might have contributed to the over-all retention of sodium and chloride (68), although with substances such as testosterone, potassium, inorganic phosphorus, and nitrogen are retained in addition to sodium and chloride, and the shifts in electrolyte balance are not nearly so rapid nor so great as those observed with desoxycorticosterone.

The retention of sodium and chloride which occurred with repeated injections of ACTH is to be contrasted with the increased excretion which was observed during a four-hour period following the single injection of 25 mg. of ACTH. Whether the increased sodium and chloride excretion observed in the short experiments reflects a preponderant secretion of 17-oxysteroids during the early phase of the increased adrenal cortical secretory activity cannot be stated at this time.

Patient M.F. with Addison's disease showed neither a significant sodium and chloride retention during four days of ACTH administration (40 mg. per day) nor a rebound sodium and chloride excretion following withdrawal of ACTH.

To determine with greater certainty that contaminating substances were not responsible for the retention of sodium and chloride, patient M.R. was given one unit of pituitrin as a substitute for 2.5 mg. of ACTH every six hours at a time when this patient was showing sodium and chloride retention under the influence of ACTH. On this regimen sodium excretion increased while the urine volume remained essentially the same, demonstrating that quantities of posterior pituitary extract much larger than those contained in the ACTH were not primarily responsible for the sodium and chloride retention observed in patient M.R. This was further corroborated

by studies on the normal male subject R.P. who was given pituitrin, two units intramuscularly every six hours, following a control no-treatment period. No retention of sodium occurred over a three-day period with pituitrin in contrast to a 40 per cent retention of sodium with ACTH containing approximately one-half this quantity of pituitrin.

These findings taken together suggest that the marked sodium retention which followed ACTH administration in the presence of adequate adrenal response was probably mediated through the liberation of desoxycorticosterone-like factors.

DISCUSSION

The ACTH preparation used in this study proved to be physiologically active as indicated by the marked hematologic and metabolic changes which were observed following a single dose of hormone as well as after prolonged administration. The fact that the magnitude of the response did not change with repeated administration of the hormone at two to three week intervals suggests that antihormone formation had not taken place in these relatively short-term experiments. Continued or repeated stimulation by ACTH or adrenal cortical hormone administration, however, tended to diminish the responsiveness of certain end organs. This condition appears to be similar to that observed by Page *et al.* (3) in hypophysectomized rats made hypertensive with ACTH and should be differentiated from inactivity due to antihormone formation.

Dougherty and White (23) established the lympholytic action of adrenal cortical secretions by demonstrating the activity of 11-17-oxysteroids in adrenalectomized mice and of ACTH in intact animals. These findings were subsequently shown to apply to man by Thorn *et al.* (70), Hills *et al.* (33), and by Darrow and Hellman as cited by Dougherty and White (21). Nordenson (49), however, failed to demonstrate any hematologic changes whatsoever following the administration of ACTH in the human.

In an earlier report (70) the authors observed that the fall in circulating eosinophils was more pronounced and more consistent following the administration of ACTH than was the change in lymphocytes. Furthermore, with continued administration of ACTH the eosinophil level remains low throughout; whereas the lymphocytes tend to rise after an initial depression. It is possible that the more precipitous and sustained fall in circulating eosinophils as compared to lymphocytes is due to the relatively small body reserve of the former in contrast to the large quantity of lymphoid tissue. Thus, with the elaboration of a given amount of hormone, it might be possible for lymphoid tissue to compensate more effectively for an initial depletion of circulating lymphocytes.

Neither a fall in circulating eosinophils nor in lymphocytes was ob-

served in patients with Addison's disease given ACTH. Compound F, however, had a very specific effect in producing these changes. Compound A and desoxycorticosterone were inactive in the dosage used.

The site and the mechanism of eosinophil and lymphoid destruction has not been established in the human. The authors' observations suggest that neither the spleen nor the thymus is responsible for the destruction of circulating eosinophils and lymphocytes. By analogy with animal work the site of lympholysis probably lies within the fixed lymphoid tissue (22).

In accordance with the findings of Dougherty and White in mice (23) the neutrophilia following ACTH administration in man was found to be partly nonspecific, the magnitude of change in patients with Addison's disease being approximately one-half that observed in normal subjects and patients with adequate adrenal reserve.

The hematologic changes observed following the injection of ACTH, characterized by a fall in circulating lymphocytes and eosinophils with a rise in neutrophils, appear to be similar to the blood picture described by de la Balze *et al.* in patients with Cushing's syndrome (7).

In the experiments reported, 17-ketosteroid excretion following ACTH increased to high normal levels and was accompanied by clinical evidence of increased androgen secretion.[13]

A five to tenfold increase in 11-oxysteroid excretion was observed, rising to levels comparable to those found in patients with Cushing's syndrome (65). Marked metabolic changes accompanied the increased excretion of these steroids.

There was a consistent increase in the urinary excretion of uric acid; whereas total nitrogen excretion rose in only one of the three patients studied. The discrepancy between the effect on the excretion of purines as opposed to that of nitrogen closely parallels the findings of Babad in rats treated with whole adrenal cortical extract (6). It also confirms the previous findings in man (30) that 11-oxysteroids tend to increase urinary uric acid irrespective of the changes in total nitrogen excretion, a fact also noted by Pincus under conditions of stress (53). Since the maximum increase in uric acid production coincides with the greatest depression of circulating lymphocytes, it appears probable that the origin of the uric acid lies in the nuclear material of the lymphoid tissue. It is unlikely that the eosinophils contribute materially to the total increase in uric acid because of their small number and relatively low content of nucleoprotein.

Changes in carbohydrate metabolism included a rise in fasting blood sugar level and liver glycogen content. The over-all increase in endogenous

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[13] It is recognized that 17-ketosteroids estimated by the Zimmermann reaction may include 3 and 20-ketosteroids as well. This is made unlikely under the conditions of these experiments by the findings of Mason et al. (47).

carbohydrate formation and decreased utilization were similar to the changes found by Ingle *et al.* (35) and Bennett and Li (9) working with forced-fed and diabetic rats respectively. The source of the additional carbohydrate in man remains obscure.

A lowered serum albumin with a variable rise in globulin has been described in patients with Addison's disease (48) and in adrenalectomized animals (32, 39). This pattern in man was reversed by the administration of whole adrenal cortical extract. In rats with intact adrenals Li *et al.* (42) have recently shown a rise in serum albumin with a reciprocal fall in globulin following the administration of ACTH. These changes are comparable to those observed in patient M.R. in the present study.

TABLE 15. ACTION OF STEROIDS ON RENAL EXCRETION OF SODIUM AND POTASSIUM

Types of Steroids	Predominant action on the kidney	
	Sodium	Potassium
Desoxycorticosterone "salt hormone"	Retention	Increased Excretion
11-17-oxysteroids "S" hormone	Increased Excretion	Increased Excretion
Androgens "N" hormone	Retention	Retention

White and Dougherty (77) reported an increase in gamma globulin and antibody titer following ACTH administration in rabbits. Li and Reinhardt (42) have failed to confirm these observations in rats. In the present study the authors obtained no significant increase in gamma globulin in two human subjects treated from four to six days with repeated injections of ACTH in spite of greatly increased 11-oxysteroid secretion.

In addition to the evidence of increased elaboration of androgens and 11-oxysteroids following ACTH administration, a rise in the secretion of desoxycorticosterone-like factors was suggested by a marked sodium and chloride retention followed by a "rebound" excretion upon the withdrawal of ACTH. There was a definite variation in the degree of sodium retention in different subjects.

It is well established that the predominant action of desoxycorticosterone on electrolyte metabolism consists of sodium retention with increased potassium excretion (72) and that the administration of 11-17-oxysteroids induces increased excretion of both sodium and potassium (69); whereas the androgens effect a simultaneous retention of both sodium and potassium along with phosphorus and nitrogen (68). On the basis of the

differential effect of these three types of steroids on the renal excretion of sodium and potassium, it should be possible to deduce the type of adrenal cortical hormone activity predominating at a given time (Table 15). For example, the retention of sodium in the presence of increased excretion of potassium indicates excess desoxycorticosterone-like action; whereas the increased excretion of both sodium and potassium is compatible only with predominant 11-17-oxysteroid action; conversely the retention of sodium and potassium suggests a predominance of androgenic activity.

It is of interest to note that following a single injection of ACTH a diuresis of sodium, potassium, and chloride was consistently observed as related to endogenous creatinine excretion. These changes suggest a predominant 11-oxysteroid effect. In contrast, the retention of sodium and chloride in the long-term experiments favors an over-all predominance of action of desoxycorticosterone-like factors. Variations in the degree of sodium retention might also be explained on the basis of these postulates.

The preparation of ACTH used in the present studies was not an homogenous protein as was the case with Li's material employed by Mason *et al.* (47), although its adrenocorticotropic activity as measured by assay on hypophysectomized animals was of the same order of magnitude as that of Li's preparation. Whereas the authors' findings of increased urinary excretion of adrenal steroids with ACTH administration are fully confirmed by the findings of Mason *et al.* using Li's preparation, the absence of any significant hematologic and metabolic changes in the latter experiments are at variance with the authors' findings. Of particular note is the complete absence of sodium and chloride retention in their studies. The apparent discrepancies between the two groups of experiments could be due to one of several possibilities:

1) That contamination in the preparation used in the authors' studies might have been responsible for the profound fall in renal excretion of sodium and chloride. Control experiments indicated that the posterior pituitary contaminants could not be indicted, although the possibility that some as yet unrecognized contaminant exerted this effect has not been ruled out. The absence of any significant response in patients with Addison's disease makes this rather unlikely.

2) That the choice of patients might have conditioned the response. It is unlikely that this was the only factor, since a positive response was observed in three types of individuals, a young normal male, a middle-aged pituitary-deficient male, and a young undernourished female.

3) That the species difference in the origin of the two extracts or the difference in the methods of preparation might have been responsible for the variations in activity.

4) That the high degree of purification of the preparation employed by Li *et al.* (41) might possibly have led to inactivation or loss of a particular portion of the activity of the original extract.

The demonstration of a multiple stimulating action of ACTH involving the three major functions of the adrenal cortex is at variance with current concepts, such as Albright's (1), allocating to ACTH the stimulation of carbohydrate-regulating factors and to anterior pituitary luteinizing hormone the stimulation of androgenic cells of both cortex and testes. The authors' observations would also tend to disprove, for man at least, the well-documented fact that in the rat (20) desoxycorticosterone-like factors are produced in the glomerulosa which is itself not under pituitary control. This might explain also why Ingle found an increased sodium loss in rats given ACTH (35), in contrast to the sodium retention noted in the three subjects given prolonged ACTH treatment.

The minimal response of patients with Addison's disease to the administration of ACTH suggests that the changes brought about by the preparation used were mediated through the adrenal cortex and did not represent a direct action of ACTH on end organs, particularly since these patients proved capable of responding to direct adrenal steroid therapy. In individuals with an intact pituitary gland it is, of course, possible that part of the effect observed following the injection of ACTH may be due to increased adrenal steroid secretion accompanying an alarm reaction provoked by the intramuscular injection itself or by any of the constituents of the hormone preparation. Conclusive proof that the changes observed are due solely to direct adrenocorticotropic action of the material injected could be obtained by a study of patients with pituitary insufficiency of recent origin only, since animal studies have shown (45) and the authors' findings suggest (Table 9) that persistence of hypophyseal deficiency for any appreciable period results in irreversible atrophy of the adrenal cortex and hence diminished response to ACTH. Reasons for believing that the changes observed in these studies were due largely to the direct action of the ACTH preparation on the adrenal cortex are:

1) The striking hematologic and metabolic changes following the injection of small quantities of the ACTH preparation (10 to 25 mg.).

2) The limited effect in normal individuals of relatively large quantities of contaminating posterior pituitary factors.

3) The relative ineffectiveness in normal subjects of the injection of specifically inactivated ACTH (43).[14]

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[14] Thirty mg. of ACTH 13-10-6 was dissolved in 7.5 cc. of 0.3 M sterile phosphate buffer pH 7. Five-tenths cc. of 0.1 N iodine was added, and the preparation was allowed to stand at 22°C. for one hour; then 3 drops of 0.3 M sodium sulfite were added to decolorize the solution. At this point the denatured ACTH settled out as a fine sus-

The availability of an active ACTH preparation capable of stimulating the three principal types of adrenal cortical activity should afford an excellent opportunity to reproduce and study the so-called alarm reaction in man (59) in its pure form.

SUMMARY

1) A single dose of 25 mg. of ACTH administered intramuscularly to more than one hundred patients and normal subjects was well tolerated.

2) Profound hematologic changes occurred following the injection of ACTH. The maximum effect was observed within four hours after a single dose of hormone and was characterized by a sharp fall in circulating eosinophils accompanied by a less spectacular and less consistent fall in lymphocytes associated with a somewhat nonspecific rise in neutrophils. Circulating eosinophils fell 74 per cent in fifty patients with presumably intact adrenals. In thirty patients with well-established Addison's disease the mean fall was only 4 per cent. A normal eosinophil response could be elicited in patients with Addison's disease given 20 mg. of Compound F; whereas Compound A and desoxycorticosterone were ineffective.

3) A single dose of 25 mg. of ACTH was followed by an increased renal excretion of sodium, chloride, potassium, and uric acid; creatinine excretion remained constant or decreased. The maximum effect appeared within four hours after hormone administration. For fifty subjects with intact adrenals the increase in uric acid-creatinine ratio amounted to 87 per cent; whereas in thirty patients with Addison's disease the mean increase was only 16 per cent. Compound F (20 mg.) administered to patients with Addison's disease elicited a mean increase in uric acid-creatinine ratio of 48 per cent; whereas Compound A and desoxycorticosterone were relatively ineffective in this respect.

4) A clear-cut differentiation between non-Addisonians and Addisonians was observed with respect to the fall in circulating eosinophils following the administration of ACTH, in contrast to the changes in uric acid-creatinine ratio which showed a considerable overlap. On the basis of these findings a simple clinical test for adrenal cortical reserve of 11-17-oxysteroids was standardized. Its interpretation and limitations are discussed.

5) ACTH was administered over a period of from four to six days to three subjects under control conditions. Hematologic and metabolic studies indicated that all known functions of the adrenal cortex appeared to be enhanced.

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pension. The material was given to a human subject by intramuscular injection, and on the following day a suspension of active ACTH 32-D was administered similarly. Response: Eosinophils showed a 65 per cent fall with the control ACTH and only 7 per cent fall with the denatured material; whereas the uric acid-creatinine ratio, which had risen 54 per cent, rose 33 per cent.

a. Increased adrenal androgen secretion was suggested by a rise in urinary 17-ketosteroid excretion.

b. An increase in 11-oxysteroid secretion appeared probable on the basis of a rise in the urinary titer. Further evidence of increased adrenal 11-oxysteroid secretion was suggested by a rise in fasting blood sugar levels with an increase in liver glycogen in the one patient in whom it was studied, by a decrease in serum inorganic phosphorus with increased urinary excretion in two subjects, and by an increase in total nitrogen excretion in only one. A fall in circulating eosinophils and lymphocytes with a rise in uric acid-creatinine ratio was taken as evidence of an increased secretion of 11-17-oxysteroids. No significant change in serum gamma globulin was observed in the two cases in which it was studied.

c. An increased secretion of desoxycorticosterone-like factors was suggested by the striking sodium and chloride retention with increased potassium excretion and a small concurrent rise in serum carbon dioxide combining capacity. Following the withdrawal of ACTH there was a "rebound" sodium and chloride diuresis, closely approximating the retention previously observed.

d. A control experiment in which ACTH was administered to a patient with Addison's disease in identical amounts under similar circumstances failed to show the changes noted above, suggesting that the adrenal cortex was essential for the response observed following ACTH administration.

6) The possibility existed that contaminating substances in the preparation of ACTH used in these studies might have acted as a nonspecific stimulant to the anterior pituitary and to the adrenal cortex. Control experiments with posterior pituitary extract, the major known contaminant, indicated that this factor could not have accounted for changes comparable in magnitude to those observed following the administration of ACTH.

ACKNOWLEDGMENTS

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The authors are indebted to Miss Mary Kascht research dietitian, for the dietary management and to Mrs. R. E. Dell and Mrs. P. H. Forsham, technical assistants, who were responsible for carrying out most of the laboratory work in this investigation.

CLINICAL ABSTRACTS

R. P., P.B.B.H. #6A169

A twenty-one-year old single male medical student in perfect health.

P.H. is negative.

P.E.—A somewhat obese young man with normal secondary sex characteristics. Blood pressure 138/90 mm. Hg, temperature 98°, pulse 80, respirations 20.

Laboratory findings entirely normal. Twenty-four-hour 17-ketosteroid excretion 8.4 mg.; 11-oxysteroid excretion 0.22 mg.

M. R., P.B.B.H. #R3654.

P.H. This forty-six year old married male had suddenly developed polyuria, polydipsia, and headache following a brief febrile episode some two years before the present admission. There was symmetrical diminution in visual fields. This was followed by loss of libido and gradual disappearance of body hair and other secondary sex characteristics and increasing weakness. There also was a history of gallbladder disease.

P.E. on previous admission one year before the present study revealed blood pressure 110/74 mm. Hg, temperature 94°, pulse 120, respirations 20. The patient was a well-developed, middle-aged white male. The skin was lemon-colored and of extremely fine texture, smooth and dry. There was complete absence of body hair, and both prostate and testes were very small. The thyroid could not be felt. The heart was small to percussion.

Laboratory findings: Average daily urine output 3000 cc., reduced by small amounts of pituitrin. Normal kidney function tests. Twenty-four-hour 17-ketosteroid excretion 2.4 mg. Visual fields showed moderate peripheral constriction. X-rays of the skull revealed a ballooning of the sella turcica, which measured 16×18 mm. There were no signs of intracranial pressure. Air encephalograms failed to show any other signs of a localizing lesion. An electroencephalogram revealed slow waves without any focus. The heart was 17 per cent below the normal average. X-rays of the gallbladder revealed cholelithiasis.

Treatment: The patient was given X-ray treatment to the sella turcica through three portals, 250 "r" per day in rotation, receiving a total of twelve treatments or 3000 "r."

Follow-up within three months revealed a decided decrease in polyuria and polydipsia. At the end of six months the urine output was approximately normal. The patient noted an increase in strength, there was a slight return of body hair, and the patient had to shave first once and then twice a week. The visual disturbance cleared up completely in short order.

The patient re-entered the hospital one year later to have a cholecystectomy and was then subjected to the studies reported in this paper. On this admission the X-ray of the pituitary fossa showed no changes from that observed a year ago. The 17-ketosteroid excretion was 1.9 mg. per day, and the 11-oxysteroid excretion was found to be 0.25 mg. per day. A Kepler-Power water test gave an A value of 17, which made this test strongly positive for adrenal cortical insufficiency. The B.M.R. was minus 8. Total cholesterol was 295 mg. per cent.

Diagnosis: Chromophobe adenoma of the pituitary with diabetes insipidus and mild anterior pituitary deficiency.

Cholelithiasis

J. W., P.B.B.H. #5A60.

P.H. This fifteen-year old girl entered complaining of weight loss of twenty-two pounds in six months, amenorrhea, anorexia, and fatigue. She had received repeated X-rays treatments for congenital hemangiomata in the past.

P.E. Blood pressure 120/70 mm. Hg, temperature 98°, pulse 76, respirations 20. A very thin girl in no distress. Skin diffusely tanned with some definite areas of brown pigmentation. There was a large hemangioma extending from the left back to the left upper abdomen. Moderate generalized adenopathy. Liver palpable one finger breadth below the costal margin. Heart small.

Laboratory findings essentially normal with the exception of a low twenty-four-hou 17-ketosteroid excretion of 2.3 mg. and an 11-oxysteroid excretion of 0.55 mg. Th B.M.R. was minus 26 per cent of normal.

Course: Although the patient showed a normal response to the ACTH test for adren cortical reserve she was given a course of desoxycorticosterone injections, 5 mg. per da On this regimen she gained weight and felt better than on interspersed periods wit placebos. On a high calorie diet, her appetite improved, and she gained weight.

Diagnosis: Anorexia
? Mild adrenal insufficiency

M.F., P.B.B.H. #K60760.

P.H. This forty-nine-year old housewife with known Addison's disease for six year entered with a story of weakness, anorexia, increasing pigmentation, and loss of pubi hair. She had been treated with three 125 mg. pellets of desoxycorticosterone acetat implanted once every year. Soon after the institution of therapy a diffuse enlargement o the thyroid was noted, her B.M.R. was found to be plus 4 per cent and she receive various types of thiouracil intermittently especially after an intercurrent bout of cardia failure three years before the present admission.

P.E. Blood pressure 180/70 mm. Hg, temperature 99.6°, pulse 71, respirations 16 A well-developed, blue-eyed, fair-haired, rather large-boned woman in no distress. Skir showed diffuse light-brown pigmentation, marked pigmentation of elbows with a vitiliginous area over the thyroid. No axillary or pubic hair. No exophthalmos or lidlag. Thyroid symmetrically enlarged, firm, two and one half times normal size. Heart not enlarged. No murmurs. Liver palpable one finger breadth below the costal margin.

Laboratory data: Only the following deviated from the normal: Hematocrit 38, white count 4,800, 34 per cent neutrophils, 60 per cent lymphocytes, 6 per cent eosinophils. Protein bound iodine 3.4 gamma per cent. B.M.R. (average of three determinations) plus 3 per cent. Twenty-four-hour 17-ketosteroid excretion 2.8 mg. Eleven-oxysteroids 0.12 mg. A standard ACTH test revealed no fall in the eosinophils and a 19 per cent rise in uric acid-creatinine ratio.

Diagnosis: Addison's disease
?Hyperthyroidism

N.B. It should be noted that a B.M.R. of plus 3 per cent represents hyperthyroidism in patients with Addison's disease who normally show a rate of minus 18 per cent of normal.

Miscellaneous Patients:

The *thirty patients with Addison's disease* presented in this paper have been followed in the medical clinic of the Peter Bent Brigham Hospital for more than one year with the exception of one case. The diagnosis was established by the finding of typical pigmentation, small heart size in the untreated stage, low 17-ketosteroid excretion, and the occurrence of intermittent typical adrenal crises in about one-half of the patients. Nearly all of these were treated by the subcutaneous implantation of pellets of desoxycorticosterone, and at the time of the ACTH administration most of them still had active implants, while a very few could no longer be considered to be deriving any desoxycorticosterone from the old sites of implantation. Supplementary whole adrenal extract therapy was discontinued in those who were receiving it at least three days before the administration of ACTH.

Fifty Non-Addisonians: A group of ten entirely normal subjects as well as forty sub-

jects with miscellaneous diseases were used. Cases suffering from renal insufficiency, hepatic insufficiency, or any known clinical state which might be associated with decreased adrenal cortical function were rejected.

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FURTHER STUDIES ON THE METABOLISM OF THERAPEUTIC DOSES OF NATURAL ESTROGENS IN HUMAN SUBJECTS*

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We have shown that our procedure for the fractionation and photometric estimation of urinary estrogens (estrone, estradiol, and estriol) (7, 8) is capable of yielding quantitative information regarding the interconversion, destruction and elimination of single therapeutic doses of the natural estrogens in (a) a normal human female, (b) a bilaterally ovariectomized human female and (c) a bilaterally ovariectomized, hysterectomized human female (9). The present report concerns estrogen studies on the human male, which were made to establish a basis for work of a similar nature on human subjects with cancer of the prostate gland. However, our data on the bilaterally orchiectomized human male should prove of broader significance since the influence of endogenous estrogen secretion, sensitive organs, cyclic phenomena, etc. which might further complicate studies of exogenous estrogen metabolism in the human female, would appear to be reduced to a minimum. The human male has served for isolation and identification studies of the urinary metabolites of massive doses of the natural estrogens in human subjects (2, 3, 4, 5) but quantitative data on the fate of single therapeutic doses of the natural estrogens in the human male are not available, to the authors' knowledge.

METHODS

Two consecutive twenty-four hour urine specimens were collected immediately following administration of a single therapeutic dose of the estrogen (pharmaceutical preparations only were used). Each specimen was extracted and prepared for chromatography essentially as described by us (7) except that the residue from evaporation of the butyl alcohol was dissolved in 100 ml. of 1 normal sodium hydroxide and diluted with distilled water to 250 ml. volume. Thirty ml. each of concentrated hydrochloric acid and toluol (Toluene, C.P.) were added and the mixture boiled under reflux for twenty minutes. After cooling under running tap water, the mixture was extracted with ethyl ether as previously described (8), except that the

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toluol was carried along in the ether extract. The estrogen content of each chromatographic filtrate fraction was calculated by means of our color correction equation with the Kober reagent (8). A correction also was made for the average 30 per cent loss of estriol by our procedure.

In those cases in which separate determinations of free (unconjugated) and total (free plus conjugated) estrogens were carried out, an isopropyl ether extraction of an aliquot of the combined forty-eight hour urine specimens was substituted for the butyl alcohol extraction. The urine aliquot was extracted three times by stirring with one-third volume of isopropyl ether. The combined ether extract was evaporated to 300 ml. volume and then extracted with 1 normal sodium hydroxide as described for the hydrolyzed ethyl ether extract (8). A second urine aliquot was extracted with butyl alcohol, hydrolyzed, and prepared for chromatography as described in this report.

We have found that the following modification of our chromatographic tube improved its performance. The sintered glass disc (medium porosity) was sealed in the chromatographic tube (13×300 mm.) approximately 10 mm. from the bottom end instead of in the inner joint of the modified adaptor.

RESULTS

Excretion of urinary estrogens following administration of single therapeutic doses of the natural estrogens to the same individual. The urinary excretion data from a healthy bilaterally orchiectomized man, aged forty-seven years, is summarized in Table 1. At least one week intervened between tests. It must be emphasized that our utilization of a color correction equation for the Kober reagent in urine with low estrogen content imposes an uncertainty factor of plus-minus 7 micrograms of estrone, plus-minus 10 micrograms of estradiol, and plus-minus 5 micrograms of estriol per twenty-four hour urine specimen (8). In view of this limitation on the accuracy of our estrogen titers, many of the percentage distribution figures in Table 1 (α-estradiol benzoate and dipropionate) can be only approximate, and in a few instances, such as that of ethinyl estradiol, are unwarranted. Furthermore, our interconversion or recovery data from the equine estrogen mixture (Urestrin gelatin capsules, Upjohn) and the equine conjugated estrogen mixture (Premarin tablets, Ayerst) cannot be utilized for any quantitative conclusions since the composition of these impure estrogenic mixtures are unknown to us. We have not included in Table 1 our estrogen excretion data from the administration of 1.92 mg. of estriol (oral) since there was no discernible conversion to estrone or estradiol. The estriol was recovered in the urine, however, in 20 per cent yield during the forty-eight hour post-ingestion period. As indicated by the data in Table 1, stilbestrol, 10.0

Table 1. Urinary Estrogen Excretion Data on a Bilaterally Orchiectomized Man Following Single Therapeutic Doses of the Natural Estrogens

Estrogen administered	Dose mg.	Urinary fraction*	Estrogen Excretion		Total	Percentage distribution
			First 24 hours	Second 24 hours		
α-estradiol (tablets)	2.0 (oral)	O	172	12	184	65
		D	60	4	65	22
		T	20	16	36	13
			252	32	285	
α-estradiol benzoate	1.66 = 1.2 D	O	20	23	43	56
		D	9	3	12	16
		T	9	13	22	28
			38	39	77	
α-estradiol dipropionate	2.5 = 1.8 D	O	11	14	25	37
		D	8	17	25	37
		T	6	12	18	26
			25	43	68	
Ethinyl estradiol	1.0 (oral)	O	3	2		
		D	0	−5		
		T	2	1		
			5	−2		
Estrone in oil	2.0	O	106	40	146	58
		D	24	12	36	14
		T	34	37	71	28
			164	89	253	
Estrogen mixture equine	10,000 I.U. oral	O	61	8	69	71
		D	9	3	12	12
		T	9	7	16	17
			79	18	97	
Estrogen mixture conjugated equine	5.0** (oral)	O	95	17	112	30
		D	130	28	158	43
		T	70	28	98	27
			295	73	368	
Stilbestrol	10.0 mg. (oral)	O	4	0	4	
		D	0	8	8	
		T	4	0	4	
			8	8	16	

* O—Estrone; D—Estradiol; T—Estriol

** Expressed as sodium estrone sulfate.

mg. orally, is not excreted in forms which react with the Kober reagent. This observation opens up promising possibilities for the study of endogenous estrogen excretion in the pregnant female under treatment with stilbestrol.

Comparison of urinary estrogen excretion following intramuscular injection and oral administration of α-estradiol. Table 2 summarizes the urinary excretion data on a normal man following treatment with a 2.0 mg. dose of α-estradiol[1] (a) by intramuscular injection (in oil) and (b) by oral ingestion (tablets). There was rapid absorption of α-estradiol by both routes as indicated by the high excretion level during the first twenty-four hours following treatment. Furthermore, absorption from the digestive tract appears to be on a higher level as judged by the excretion levels, in contradistinction to the generally recognized low level of estrogenic effect by this route. Obviously there are other factors besides absorbability which govern the tissue response. Inactivation by degradation to less estrogenically active forms does not seem to be remarkably different for the two routes, as judged by the lack of any significant variation in the percentage distribution values.

TABLE 2. COMPARISON OF URINARY ESTROGEN EXCRETION FOLLOWING INTRAMUSCULAR AND ORAL ADMINISTRATION OF α-ESTRADIOL TO A NORMAL MAN

Estrogen administered	Dose mg.	Urinary fraction*	Estrogen excretion: First 24 hours	Estrogen excretion: Second 24 hours	Total	Percentage distribution
α-estradiol in oil	2.0	O	141	34	175	55
		D	85	15	100	32
		T	24	17	41	13
			250	66	316	
α-estradiol (tablets) oral	2.0	O	234	23	257	65
		D	67	14	81	20
		T	33	26	59	15
			334	63	397	

* O—Estrone; D—Estradiol; T—Estriol.

Comparison of urinary estrogen excretion data following administration of single therapeutic doses of the natural estrogens to male patients with and without extensive liver damage. Some data from a preliminary study

[1] The α-estradiol in oil used in this work was kindly furnished us by the Schering Corporation.

of the effect of extensive liver damage on the metabolism of therapeutic doses of the natural estrogens in human subjects is presented in Table 3. In these studies the two consecutive twenty-four hour urine specimens were

TABLE 3. COMPARISON OF URINARY ESTROGEN EXCRETION FOLLOWING ADMINISTRATION OF SINGLE THERAPEUTIC DOSES OF THE NATURAL ESTROGENS TO MALE PATIENTS WITH AND WITHOUT EXTENSIVE LIVER DAMAGE

Case no.	Estrogen administered	Hydrolysis	Urinary fraction*	Estrogen excretion per 48 hrs.		Percentage distribution
1†	2.0 mg. estrone in oil	No	O	158		69
			D	70		31
			T	0		0
					228	
1	2.0 mg. estrone in oil	Yes	O	190		66
			D	82		28
			T	17		6
					289	
1	2.0 mg. α-estradiol (oral)	No	O	538		71
			D	202		26
			T	22		3
					762	
1	2.0 mg. α-estradiol (oral)	Yes	O	478		69
			D	178		25
				42		6
					698	
2‡	2.0 mg. estrone in oil	No	O	6	First 24 hr. specimen only	
			D	2		
			T	0		
2	2.0 mg. estrone in oil	Yes	O	144		46
			D	69		22
			T	103		32
					316	

* O—Estrone; D—Estradiol; T—Estriol.
† Extensive liver damage (Laennec's cirrhosis).
‡ Bilaterally orchiectomized male with no evidence of liver damage.

combined and the total forty-eight hour estrogen excretion titers before and after hydrolysis were determined, using separate aliquots of urine. Thus we have an index to the extent of estrogen conjugation as well as interconversion and elimination in the presence of liver damage. The seeming paradox

in the α-estradiol study of greater recovery of excreted estrogens before than after hydrolysis arises no doubt, in part at least, from the destructive features of the hydrolytic process. For comparison, we have included data from a similar study on a patient with bilateral orchiectomy for cancer of the prostate, but with no clinical evidence of liver damage. In this study the first twenty-four hour urine specimen was first extracted with isopropyl ether and then re-extracted with butyl alcohol. Each extract was treated as described under "Methods."

(A) Estrone (1000 micrograms)

Percentage of Estrone eluted by successive volumes of 20% methanol-benzene													
20 ml. fractions				10.0 ml fractions		10.0 ml benzene	10.0 ml. fractions					20.0 ml. fractions	
1	2	3	4	5	6	7	8	9	10	11	12	13	14
0	7.5	37.5	27	9	6.5	4.5	2.5	3.0	1.2	0.9	0.7	1.4	1.2
Estrone eluted 87.5%							Estrone eluted 10.9%						
Estrone eluted 92%													

(B) α-estradiol (1000micrograms)

Percentage of α-estradiol eluted by successive volumes of 2.0% methanol-benzene												5% methanol benzene	
40 ml. fractions		20.0 ml. fraction	10.0 ml. fractions								20.0 ml. fraction	100.0 ml. fraction	20.0 ml. fraction
1	2	3	4	5	6	7	8	9	10	11	12	13	14
0	0.7	1.3	1.9	2.4	3.3	3.3	4.2	4.8	5.0	5.0	11.0	51.0	1.2
Estradiol 2.0%			100 ml. addition 2% methanol-benzene = 41.0%									51.0	1.2

Fig. 1. Distribution of Crystalline Estrogens Added to Phenolic Residues from 24-Hour Urine Specimens (man) Just before Chromatography (A) Estrone (1000 micrograms), (B) α-estradiol (1000 micrograms).

Distribution of crystalline estrogens added to residues from twenty-four hour urine specimens (men) just before chromatography. We previously have shown by chromatographic filtrate distribution studies (1) that our liquid chromatogram technique accomplishes satisfactory dispersion of ternary mixtures of crystalline estrone, α-estradiol, and estriol so that it may be utilized for quantitative fractionation of estrogen mixtures. We now report two chromatographic distribution studies of single estro-

gens (estrone and α-estradiol) in the presence of the essentially estrogen-free phenolic-like residue from twenty-four hour urine specimens from men. Figure 1-A shows the percentage distribution of 1000 micrograms of crystalline estrone in a liquid chromatogram which was extensively developed with consecutive additions of 2 per cent methanol-benzene. The presence of the phenolic residue appears to impose no unusual alteration in the customary distribution pattern. That is, the first 20 ml. filtrate fraction is estrogen-free and the succeeding 80 ml. contain 87.5 per cent of the estrone which was added to the phenolic-like residue, whereas our routine practice of employing 100 ml. of 2 per cent methanol-benzene plus 10 ml. of benzene elutes 92 per cent of the added estrone. Perhaps the diffuse trailing boundary which is typical of most chromatograms accounts for the persistence of very small percentages of eluted estrone beyond the 110 ml. mark. Figure 1-B shows the distribution pattern for 1000 micrograms of α-estradiol in the presence of a phenolic-like residue from a twenty-four hour human male urine specimen. The first 100 ml. of 2 per cent methanol-benzene eluent contained only 2 per cent of the α-estradiol. Successive 10.0 ml. volumes of the same eluent (for a total of 100 ml.) contained surprisingly small amounts of α-estradiol (2 to 5 per cent). However, the feasibility of employing more extensive elution with the 2 per cent methanol-benzene eluent, i.e., beyond the prescribed 100 ml., with the object of obtaining more strictly quantitative separation of estrone and α-estradiol appears contraindicated. We routinely use aliquots of our phenolic-like residues in order to keep the suspected estrogen content of any one fraction less than 500 micrograms.

DISCUSSION

Evidence compiled in our studies on separate consecutive twenty-four hour urine specimens following single therapeutic doses of the natural estrogens seems to indicate that both the oral and intramuscular routes are effective in delivering estrogens to the circulatory system. The conjugation of α-estradiol preparations for intramuscular injection so profoundly reduces the rate and extent of elimination of estrogenically active forms that their estimation by our procedure, in 1 to 2 mg. doses, is of questionable value. It is surprising in this respect that the administration of 1.0 mg. of ethinyl orally leads to no measurable recovery in the forty-eight hour urine, of estrone, estradiol, or estriol.

Although we can draw no conclusion regarding interconversion from our orally administered estrone preparations because of their indefinite composition, there is unmistakable evidence of their ready absorption in both free and conjugated forms. It must be remarked that the high urinary titer of the estradiol fraction of the conjugated estrogen mixture (Premarin)

gave an atypical initial color (pink) with the Kober reagent. This same atypical Kober color has been obtained after subjecting aqueous solutions of the tablets to our procedure for fractionation and photometric estimation of urinary estrogens. It is similar to the initial Kober reaction obtained with equilin and equilenin and may therefore indicate the presence of these compounds in the estradiol fraction. Of the pure estrogen preparations investigated, only α-estradiol and estrone in oil yielded amounts of excreted estrogens suitable for fractionation and photometric estimation by our procedure.

Our preliminary observations on the capacity of the human male to metabolize exogenous estrogens in the presence of extensive liver damage clearly indicate that there is no impairment of the capacity for reversible estradiol to estrone interconversion. There is greatly reduced capacity to convert administered estrone and α-estradiol to estriol and almost complete failure of the capacity to conjugate these estrogens. As indicated by the data in Table 1, the excretion levels for intramuscularly injected estrone fall within normal limits, whereas the excretion levels for orally ingested α-estradiol appear to be from 2 to 3 times greater. These observations of exogenous estrogen metabolism in the presence of extensive liver damage in the human male in general parallel those of Schiller and Pincus (6) in their work with partially hepatectomized rats. However, they reported that the capacity to conjugate estrogens is not present in the normal male rat under any circumstances. Glass *et al.* (1) have reported an almost complete loss of capacity for conjugation of exogenous estrogen by male patients with extensive liver damage but have also reported an almost (83 to 86 per cent) complete recovery of estrogen on an activity basis.

SUMMARY

The human male metabolizes therapeutic doses of the natural estrogens in a manner similar to the bilaterally ovariectomized hysterectomized human female. Estriol apparently is not converted to estrone or estradiol, estrone and α-estradiol are partially interconvertible, and injection of either leads to excretion of estriol also. The nature and amount of the estrogen excreted is influenced by (a) the vehicle in which the hormone is administered, (b) the route of administration, and (c) the nature of chemical conjugation of the administered hormone. Extensive liver damage does not prevent the reversible interconversion of estradiol and estrone but does greatly reduce the conversion of either to estriol and almost completely eliminates conjugation of the estrogen.

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CYTOLOGICAL CYCLE OF THE URINARY SEDIMENT AND ITS PARALLELISM WITH THE VAGINAL CYCLE

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BY STUDYING vaginal smears, Stockard and Papanicolaou (7) were the first to establish the existence of cyclic changes in the desquamation of the vaginal epithelium in the guinea pig. Subsequently, many workers have extended these observations to other organisms, including the human, and have developed various technics for obtaining, fixing and differentially staining vaginal smears. Papanicolaou and Marshall (4, 5) have recently studied smears of the urinary sediment and were able to identify cancerous cells in cases with malignancy of the genito-urinary tract. Biot and Beltran (2) studied the relationship between ovulation and the number of cells in smears of the urinary sediment. In related studies, they found an increase in the number of cells in such smears from patients with prostatic cancer who were treated with estrogenic substances. Cifuentes Delatte (3) described a mucous zone in the vaginal trigonum of women, the structural characteristics of which were similar to the vaginal mucosa. This zone was found to undergo a series of changes with respect to age.

The purpose of this paper is to describe a method which we have developed for making satisfactory smears of the urinary sediment, and to describe the characteristics of such urinary smears in the various phases of the sexual cycle of women. These urinary studies have been correlated with vaginal smears obtained simultaneously from the same patients.

MATERIAL AND METHODS

The first morning urine specimen was centrifuged and the supernatant fluid discarded. The sediment was then spread with a wire loop on a glass slide and fixed before complete drying occurred. As the study progressed, the method of obtaining the urinary sediment was simplified by eliminating centrifugation. The morning specimen was filtered through ordinary filter paper. When all but a small quantity of urine had been filtered, the paper was pierced with a loop in order to empty the funnel completely. The internal surface of the filter paper was then rubbed with a loop and the material thus obtained smeared on a slide. While the slide was still damp it was immersed in the fixing fluid.

The staining technic described by Shorr (6) was used. The slide was

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dipped in alcohol-ether for a few minutes and then stained with the single differential staining solution for three minutes. The slides were then washed in running water and allowed to dry.

In order to determine the source of the cells found in the urine sediment in several patients two methods have been carried out. One was to obtain the urine directly by introducing a catheter into only the meatus, in such a way as to collect the urine of all the urinary tract including the anterior urethra. It is important that the catheter should not be introduced more than 1 centimeter into the urethra in order to collect cells from the anterior urethra.

The other method was to introduce into the anterior urethra, after carefully washing the region, a drop of a mixture of equal parts of glycerine, alcohol and physiologic sodium chloride solution by means of a pipette and to recover that drop following a slight massage of the urethra. The drop was fixed and stained by the technic previously described. The smears thus obtained show the typical cells that will be described in this paper (Fig. 16).

The results obtained with the first method indicate that the cells in the urine sediment arise from the urinary tract and are not vaginal cells washed out by the urinary stream. The second method shows that at least some of the cells which appear in the smears arise from the anterior urethra.

RESULTS

Smears of the urinary sediment are composed of the following elements:

(a) Crystals and occasionally cylinders.
(b) Bacteria.
(c) Leukocytes and occasionally erythrocytes.
(d) Mucus.
(e) Epithelial cells, the morphology and staining properties of which are similar to those of the vagina.

Changes in these elements were noted in the following conditions:

Normal prepuberal girl: The urinary smear is characterized by the absence of mucus and leukocytes and by the scarcity of small round cells. These small round cells have large nuclei similar to the deep "spinous cells" of the vagina (Fig. 1).

Normal adult woman: Urinary smears from adult women usually contain many large cells. Their nuclei are small, round and dark. The cytoplasm stains pink or pale blue. These cells closely resemble the karyopyknotic and cornified cells of the vagina. In addition to this cell type, there are a few small, oval cells probably originating from the ureteral lining and from the kidney pelvis. There are also a few hooked or elongated cells which might have their origin in the vesical epithelium (Fig. 2). The smear

also contains mucus and leukocytes. These latter components have been found on occasions when they were also found in the vaginal smear (Fig. 3).

Menopausal women: The smear of the urinary sediment from menopausal patients shows chiefly two kinds of cells. The first type of cell is a small, round cell containing a medium-sized, blue-staining nucleus. The second type is a larger cell, similar to that seen in normal, adult women except that it is slightly smaller in size and does not stain pink. Mucus and

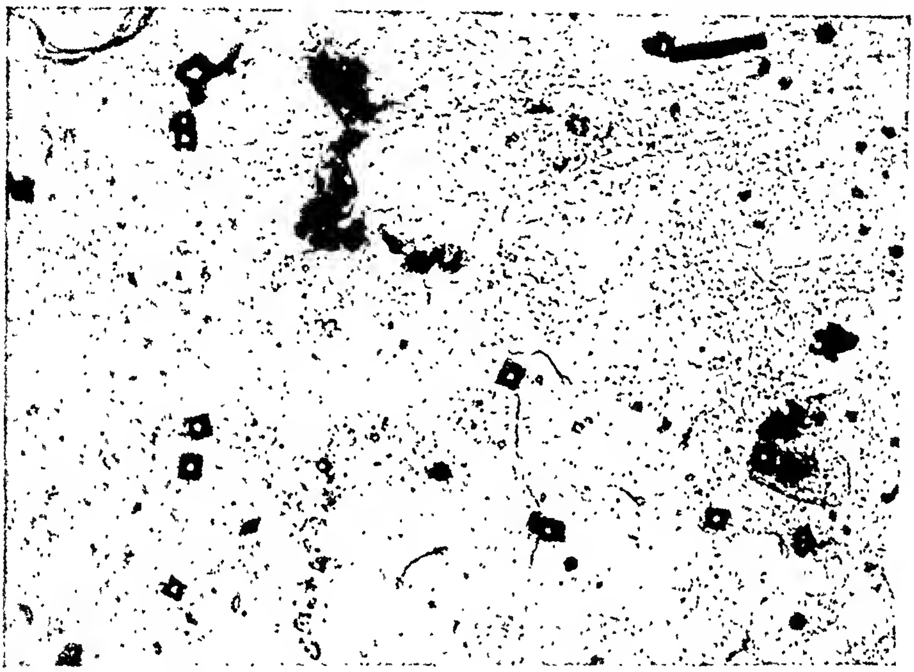

FIG. 1. Urinary sediment smear of a normal prepuberal girl.

leukocytes are encountered in variable amounts (Fig. 4). As a whole, the sediment smear in menopausal patients is very similar to the vaginal smear (Fig. 5). In both smears, many kinds of transitional cells ranging from the estrogenic type to the atrophic type are found.

Primary amenorrhea: These cases of primary amenorrhea were similar to those described by Albright (1) and by Varney (8) and others and were characterized by ovarian agenesis. The urinary sediment smear contained very few cells, and most of these were similar to the deep "spinous cells" of the vagina. The smears also contained mucus and leukocytes (Fig. 6). Vaginal smears (Fig. 7) prepared simultaneously from the same patients showed on the whole the same features that were found in the sediment smear.

Women treated with estrogens: A patient with ovarian agenesis, proved by biopsy examination of the ovaries, was given estradiol benzoate (120 mg.) by implantation. The urinary sediment and vaginal smear of this pa-

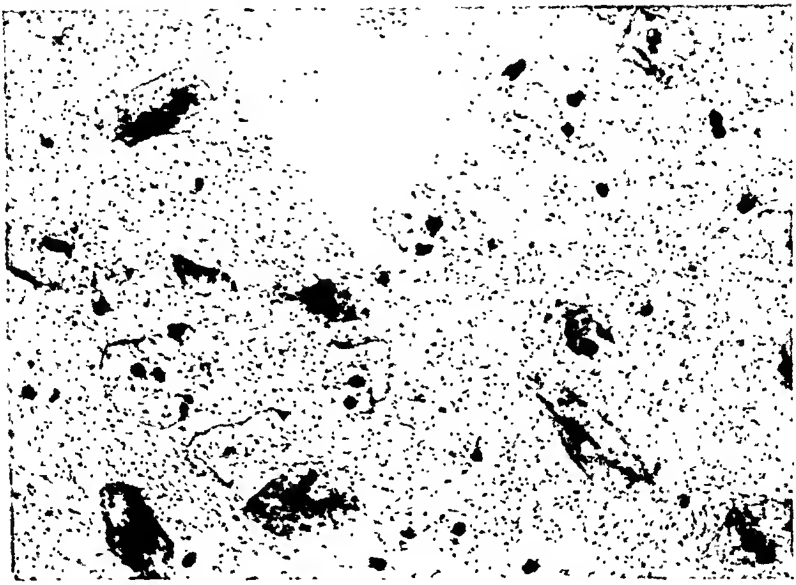

FIG. 2. Urinary sediment smear of a normal adult woman.

tient before estrogenic therapy are shown in Figs. 6 and 7, respectively. Four days following implantation of the estrogen, the number of cells in the smear was appreciably increased. The size of the cells was greater and their medium-sized nuclei stained more intensely than before treatment.

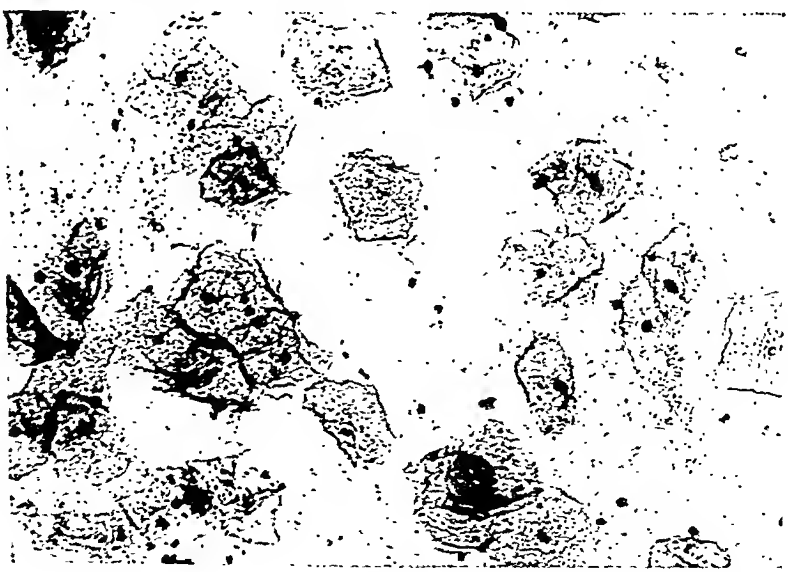

FIG. 3. Vaginal smear of the same normal adult woman (Fig. 2).

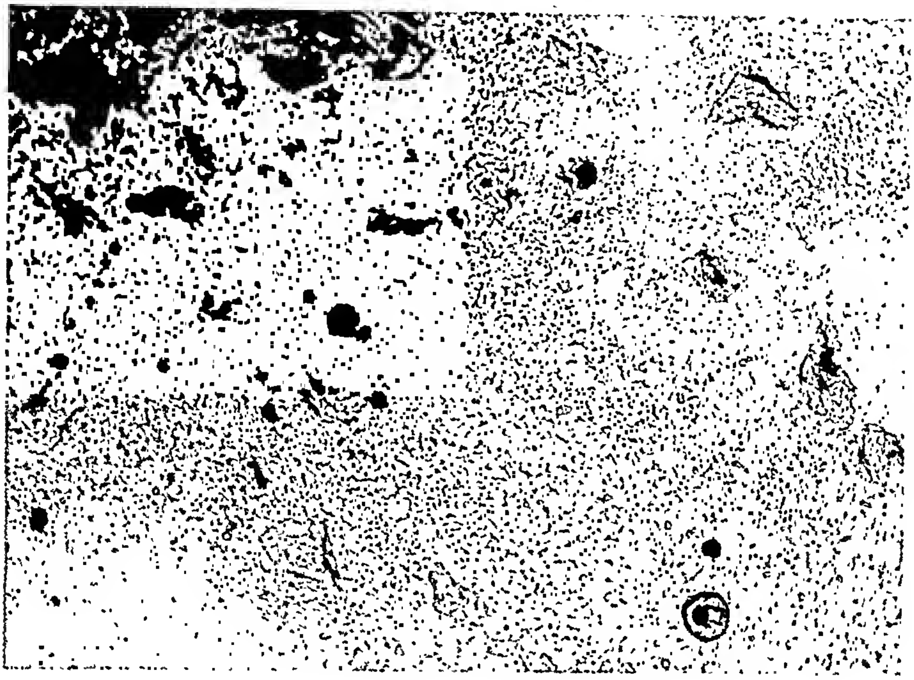

Fig. 4. Urinary sediment smear of a woman during menopause.

Mucus and leukocytes were also present in increased amounts (Fig. 8). Ten days after implantation mucus and leukocytes had disappeared. The primitive cells had been replaced by a greater number of large pink-staining cells containing small nuclei (Fig. 9). Vaginal smears obtained simultane-

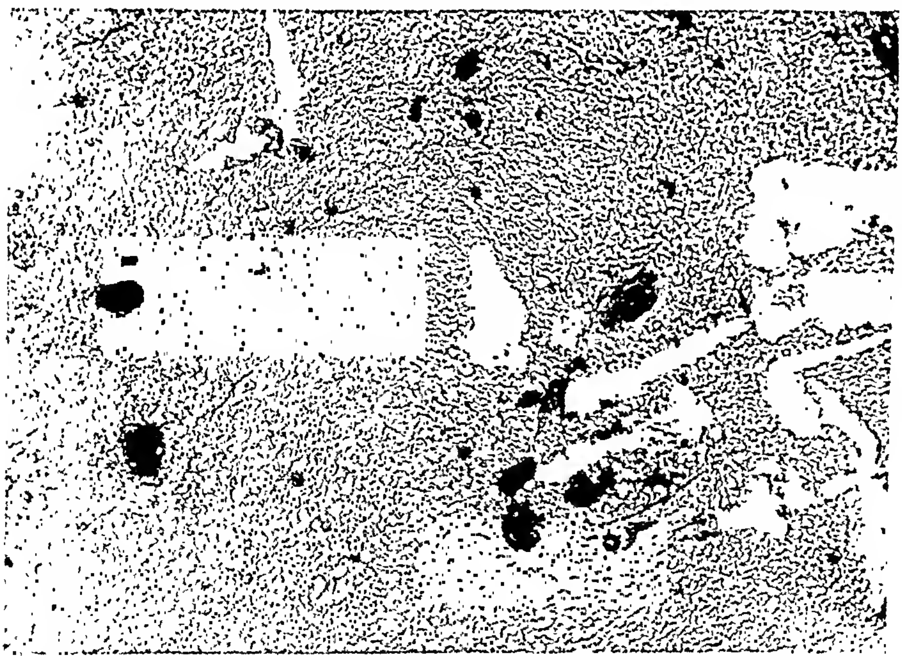

Fig. 5. Vaginal smear of the same woman during menopause (Fig. 4).

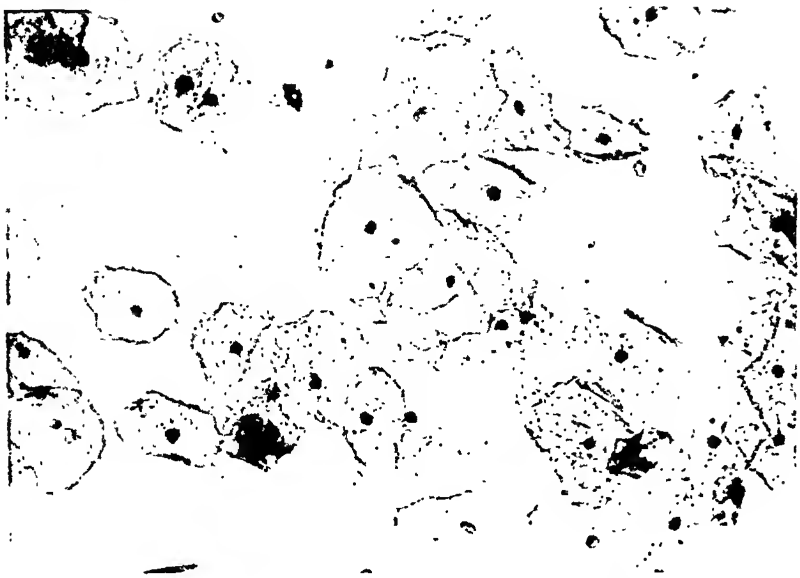

Fig. 14. Vaginal smear of the same ovariectomized patient, after treatment with large doses of estrogens (Fig. 13).

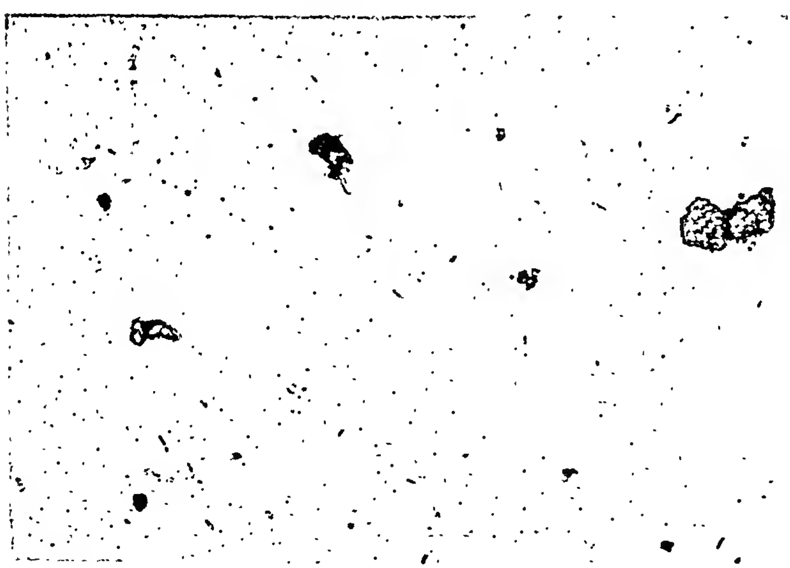

Fig. 15. Urinary sediment smear of a normal adult man.

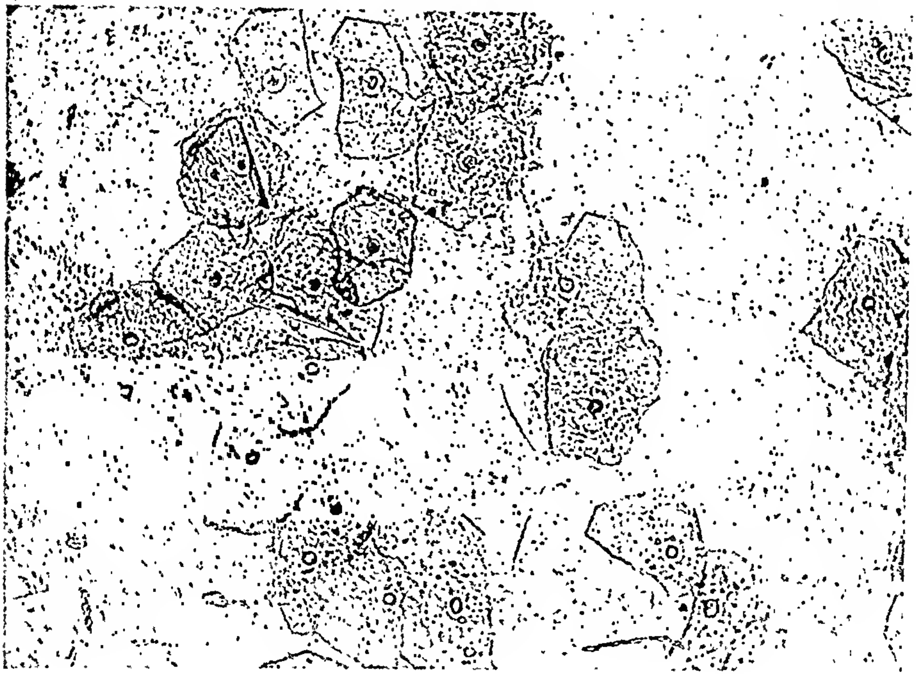

FIG. 12. Vaginal smear of the same ovariectomized patient, after treatment with large doses of estrogens (Fig. 11).

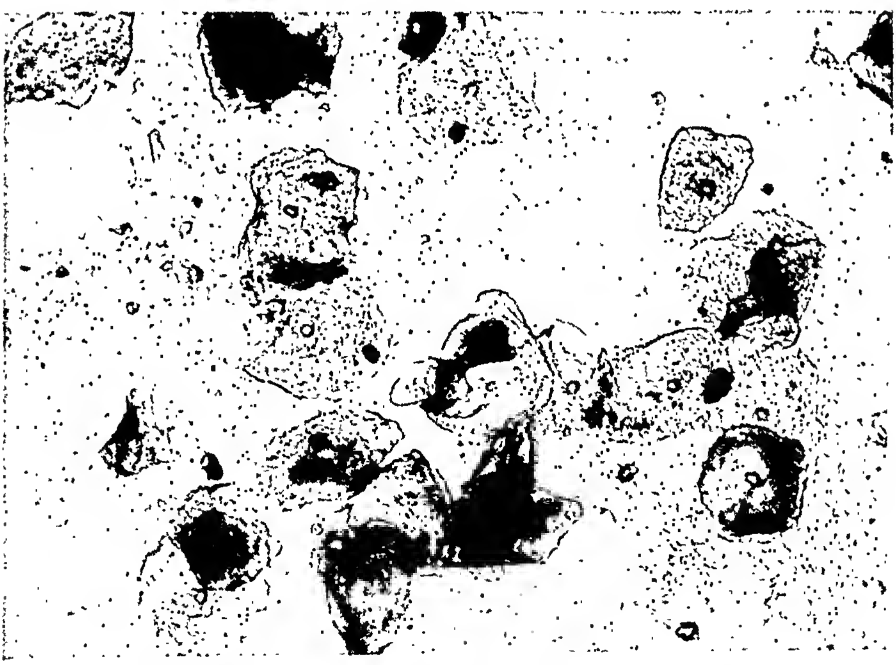

FIG. 13. Urinary sediment smear of an ovariectomized patient, after treatment with large doses of estrogens.

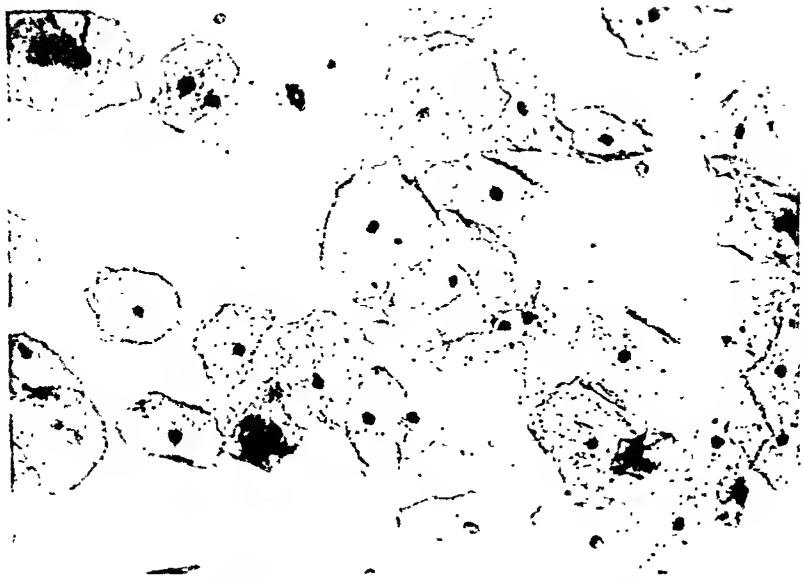

FIG. 14. Vaginal smear of the same ovariectomized patient, after treatment with large doses of estrogens (Fig. 13).

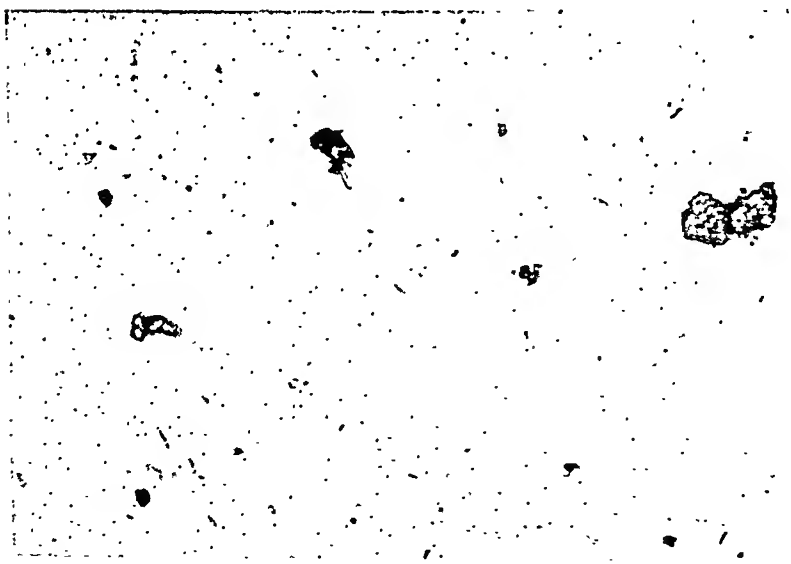

FIG. 15. Urinary sediment smear of a normal adult man.

FIG. 16. Smear obtained from the anterior urethra of a normal woman. Typical cornified cells of different sizes. Large basophilic cells, some with pyknotic nuclei. Small cells similar to the ones of the deep layers of the vagina.

CONCLUSIONS

1) A simple, rapid method adaptable to office or clinic practice, of obtaining smears of the urinary sediment in women is described. The advantages with respect to speed and convenience of the patient of the urinary smear over the vaginal smear are obvious.

2) Characteristic changes in morphology and in numbers of the cells in the urinary sediment were related to various phases of the sexual cycle of women.

3) The changes observed in the smears of the urinary sediment were found to parallel those seen in vaginal smears obtained simultaneously from the same patients.

4) The effect of estrogens on the urinary smear was found to be the same as the effect on the vaginal smear, as indicated by studies on castrated patients or on patients with primary amenorrhea.

ACKNOWLEDGMENT

We wish to express our gratitude to Dr. Ephraim Shorr for his valuable suggestions about this paper.

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SOCIAL AND PSYCHOLOGICAL READJUSTMENT OF A PSEUDOHERMAPHRODITE UNDER ENDOCRINE THERAPY*

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THE intersex individual has always been a problem to himself and to his social group. Dr. Hugh H. Young's fascinating monograph (8) covering all angles of the problem is a standard reference on the subject.

Hermaphrodites are classed as pseudohermaphrodites (male or female) and true hermaphrodites (those who possess functional gonads of both sexes). We are here concerned with a male pseudohermaphrodite, that is, an individual who possesses male gonads, but whose external genitalia and other secondary sex characteristics are either of a female or of a mixed male and female pattern. Such individuals are usually brought up as girls because of the absence of penile and scrotal development at birth. At puberty partial penile or scrotal development may occur and puzzle the person thus afflicted.

The psychological pattern of these patients may be either male or female; the majority of male pseudohermaphrodites reported in the literature had female psychological patterns and insisted on the removal of the external genitalia, which interfered with their courtship, marriage and a full expression of their feminine drives (2–7). Our patient, however, although brought up as a girl, had a strong male psychological pattern and could not make adjustments in the female role. When his true sex was determined by exploratory laparotomy at age 21, and proper therapy initiated there was marked improvement in the psychological status of the patient.

CASE REPORT

Case History: W.D. age 21, single, was first seen on September 23, 1944 because of primary amenorrhea, lack of energy and endurance, attacks of tachycardia, vertigo and allergic tendencies.

Family History: One brother two years older than the patient was operated on several times for hypospadias; the mother, a nervous individual, was preeclamptic with her last pregnancy; two male maternal cousins (twins) are effeminate and have homosexual tendencies.

Past History: The patient had the usual diseases of childhood and numerous allergic manifestations. Previous operations: a left herniotomy was performed in 1938, a ton-

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sillectomy in 1944 and a giant cell tumor of the right humerus was removed in 1941.

Physical Examination: During the first examination the findings were as follows: height, 71 inches; weight, 120½ lbs.; pulse 84; blood pressure 120/76 mm.Hg.; measurement from crown of head to symphysis pubis 34 inches; from symphysis to base 37

FIG. 1. Patient W.D. Age 21. Before therapy. Height 71 inches. Weight 120½ pounds.

inches (Fig. 1). A mild systolic murmur was heard in the mitral area. The sternum was deformed, showing evidence of an old rachitic process.

The abdominal findings were normal.

The pelvic examination revealed the presence of an enlarged clitoris measuring 4½ cm. in length and 5½ cm. in circumference; the urethra was at the base of the clitoris (Fig. 2). There was no scrotum; the labia were somewhat shrunken. The vaginal opening admitted one finger and measured 8 cm.; no pelvic organs could be palpated. The rectal examination did not reveal any evidence of a prostate. There was no evidence of testes either in the inguinal canal or in the labia majora. The pubic hair was scanty and of a feminine distribution; there was scanty axillary hair; there was no evidence of hirsuties on the face, trunk or extremities. The voice was of a fairly deep range.

Although the patient had been previously treated with estrogens by the family physician, she strongly resented attempts to induce feminization.

Two years previously the patient developed an emotional interest in a girl and began to feel the urge to assume a masculine role in this relationship. At that time she became aware of the presence of an enlarged clitoris. This discovery produced a psychic shock and the patient began to experience palpitations, dizzy spells, tinnitus, sudden blackouts, headaches, anorexia and insomnia.

Fig. 2. Patient W.D. Note enlarged clitoris and vaginal orifice.

Because of the patient's strong wish to be recognized as a male, it was decided to carry out bio-assays, determination of 17-ketosteroids and vaginal smears in order to determine the patient's true sex. The results of these studies were as follows: 1) Bio-assays of urine: The estrogen content in a 24-hour specimens was less than 5 m.u./L (normal, 5 to 60 m.u./L).[1] The pituitary gonadotropin content was 12.4 m.u./L (normal, 0 to 50 m.u./L).[2]

[1] Estrogen. A 24-hour specimen of urine was hydrolyzed with 50 cc. HCl, extracted with 250 cc. of benzene and the residue taken up in sesame oil; $\frac{1}{4}$ cc. was injected daily for

2) The 17-ketosteroids in urine measured 4.5 mg. per 24-hour specimen (normal for women, 4 to 12 mg.: normal for men, 7 to 14 mg.)[3]

3) Dry vaginal smears showed an occasional small squamous epithelial cell of an infantile type.

Bio-assay findings in other male pseudohermaphrodites have varied considerably. Novak (4) found the presence of estrogens, but no pituitary gonadotropin; Rubovits *et al.* (5) reported negative estrogen content, but positive pituitary gonadotropin; Mishell (3) and Witschi (7) found normal urinary estrogens and low pituitary gonadotropin in their patients prior to bilateral orchidectomy and the reverse after the operation. All the above mentioned patients had female psychological patterns, although at operation there was no evidence of the presence of uterus, tubes or ovaries. Our patient, however, had no evidence of estrogen production, as evidenced by urinary bio-assay and vaginal smears, nor was these any evidence of feminine tendencies or mannerisms.

Psychiatric conferences including analysis of dream material, carried out through the courtesy of Dr. Bernard Goldberg in order to determine the dominant psychosexual makeup of the patient, pointed conclusively to a predominantly masculine type of personality. The ambitions for the most part were for masculine pursuits—to be a physician, a pilot or radio operator. The patient had shown inventive ingenuity and dexterity in designing and modeling. The patient was valedictorian of her class in high school, was head of the class at Teacher's College and took a special course in engineering. In all studies, work and games the patient was always the leader of the group. The advice of the psychiatrist was to assist the patient in the strong wish to promote development of the male secondary sex characteristics and to assume the male status, which would be in harmony with the psychological makeup.

The actual evidence of the true sex of the patient could be determined only by an exploratory laparotomy with the histological section of the gonads. Since a laparotomy involves a certain amount of risk, it was decided to carry out a biological test, according to the recommendation of Diaz (1). In his report of a case of pseudohermaphrodism in a child aged 4, a therapeutic test was carried out consisting of the administration of chorionic gonadotropin in a dose of 500 R.U. twice a week for five weeks. The principle of this test is based upon the well known fact that the chorionic gonadotropin stimulates the interstitial cells of the testes and results in the production of androgens. Androgens, secreted by the testes, cause an increase in the size of the male external genitalia. This result was obtained in the case reported by Diaz and helped in the determination of the patient's sex. After conferring with our patient, it was decided to duplicate this therapeutic test using larger dosages, because of the patient's more advanced age. A short course of therapy was initiated and carried out over a period of six weeks using from 1000 to 1500 I.U. of chorionic gonadotropin every other day by intramuscular injection. At the end of this course of therapy the clitoris became engorged and somewhat thickened; the vagina also became more elastic, roomy and moist. This suggested that male gonads were present somewhere in the body and responded to stimulation with chorionic

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3 days into adult castrated mice; readings were made by means of vaginal smears.

[2] Pituitary gonadotropin. The Venning and Brown modification of the original Zondek technic of alcohol precipitation and ether extraction was used; the residue was taken up in water and injected into immature mice.

[3] The determination was carried out by Dr. Konrad Dobriner of the Memorial Hospital, New York City, using the Callow modification of the Zimmerman test with the absolute alcohol method.

gonadotropin. The patient reported having erections from two to three times per week. The patient, however, was dissatisfied with the progress and especially with lack of general constitutional stimulation and insisted that the therapy be changed to substitution therapy with testosterone compounds. Although all indications pointed to a basically male individual, it was decided to obtain final proof by exploratory laparotomy. The patient was subsequently operated on at the Brady Urological Institute by Dr. H. H. Young and Dr. H. J. Jewett and the following report was made: "Examination of the pelvic cavity disclosed an infantile testis on each side attached to the posterolateral pelvic wall by a vascular pedicle about 5 to 6 cm. long. Each testis was markedly hypoplastic, smooth, soft and measured about 6 cm. in its greatest diameter. There was no sign of ovotestis or ovarian tissue. A piece of the left testis was removed for biopsy. No Müllerian duct remnants were seen, and nothing identifiable as tubes or rudimentary uterus."

Pathological report: "The specimen consists of one piece, which is a biopsy of a left testicle weiging one gram. Microscopic: Section discloses incompletely developed seminiferous tubules containing chiefly Sertoli cells. There is no evidence of spermatogenesis; there is marked hyperplasia of the interstitial cells. The peripheral portion of the section disclosed a dense connective tissue capsule. There was no evidence of ovarian tissue in the section."

Following the operation the patient received methyl testosterone orally in a dose of from 30 to 40 mg. per day for one month. This improved the patient's vitality and spirits to a great extent and when therapy was temporarily discontinued, the patient experienced a sense of letdown and asked permission to resume the therapy. In order to achieve rapid progress in the growth and development of the secondary sex characteristics, the therapy was changed to intramuscular injections of testosterone propionate in a dose of from 100 to 150 mg. per week. There was a further increase of pubic hair and muscular development but growth of the genitalia was not very marked; however, the rectal examination revealed the presence of a small prostate. For the sake of convenience and economy, a change was made to oral medication, using methyl testosterone linguets especially prepared for sublingual use, because of the rapid absorption and utilization by this route. From 20 to 30 mg. per day was sufficient to equal the effect produced by the previous therapies. Oral and injection therapies were periodically interchanged and resulted in a gain in weight of 23 pounds, deepening of the voice, appearance of light fuzz on the upper lip, and an increase in muscular development. There was also a definite effect on the external genitalia; the clitoris gradually increased in length and circumference; there was an increase in pubic hair and a marked improvement in vitality and mental outlook. Erections occurred from two to three times a week and a yellowish emission was noted on several occasions.

For the sake of convenience, it was decided to change the therapy to pellet implantation, and on October 14, 1945, six testosterone pellets (450 mg.) were implanted. Further progress occurred in genital and muscular development and in the deepening of the voice. Although two pellets were later expelled, the favorable effects lasted four months, at the end of which time evidence of depletion was noted, such as a sense of physical exhaustion, numbness of arms and legs, vertigo, and a decrease in the number of erections. The patient was advised to resume therapy with linguets in a dose of 15 mg. daily and to report for another implantation. The second implantation was carried out on March 4, 1946, and 375 mg. of testosterone were implanted. At this time the patient definitely decided to change the legal status from a female to that of a male and to assume male attire. There were no difficulties in changing the legal status or the attire from the fem-

inine to the masculine, nor were there any serious difficulties in being accepted socially in the community as a male. The transition from female to male attire was not too obvious, because the patient always wore strictly tailored suits and mannish hair comb; the skirt was gradually changed to slacks and a $\frac{3}{4}$ coat; therefore, the change to man's

FIG. 3. Patient W.D. After testosterone therapy.
Height 71 inches. Weight $151\frac{1}{2}$ pounds.

clothes was not too abrupt. The patient expressed great relief and satisfaction in the change so ardently wished for in the previous two years.

One more step remained to be achieved: a plastic operation was needed to free the fibrous bands which bound the clitoris-penis and prevented its development and free movement. This operation was carried out in May, 1946.

The effects of the last implantation were maintained by the methyl testosterone linguets in a dose of 20 mg. per day. There was further deepening of the voice, further gain in weight ($15\frac{1}{2}$ pounds) and an increase in the length and circumference of the penis (Figs. 3 and 4). There was also the development of a small prostate, the size of a walnut; no prostate was felt on original examination, nor was it noted by the surgeons during the laparotomy.

FIG. 4. Patient W.D. After testosterone therapy and pelvic laparotomy. Note increase in pubic hair and increase in the size of the penis.

Psychologically there was great improvement in emotional stability, and a change to a cheerful and hopeful outlook on life. The patient now feels as if he has come into his own fold and station in life, thereby justifying our decision to cooperate with his wishes to change the legal and social status as well as to enhance the masculine secondary sex characteristics.

His increased physical and mental energies are stimulating him now to further studies and achievement and it is fully expected that he will continue to make progress and important contributions to science. He is now practically free from the original complaints of attacks of tachycardia, spasm of the throat and black outs. These symptoms were present two years prior to initiation of therapy and during the early stages of treatment.

In November 1946 it was decided to change the therapy once more from oral medica-

tion to pellet implantation for the sake of convenience. Eight pellets, each containing 75 mg. were implanted at that time.

The patient's voice is now of a decidedly low masculine range and he reports a great improvement in energy and endurance in his work and studies. There is an increasing sense of security and social adjustment; emotional adjustment is yet to be achieved, since the patient feels somewhat insecure in his new position as a male.

SUMMARY

This case report deals with an intersex individual, aged 21, brought up as a female, who failed to develop the normal secondary characteristics of one sex at puberty, but whose psychological makeup was definitely of a masculine pattern.

He was fairly content until the age of 19, when he developed a strong attachment for a girl and at the same time became aware of an enlarged clitoris. This discovery was somewhat of a shock and produced an emotional conflict resulting in symptoms of anxiety neurosis, which lasted for over two years until a thorough investigation revealed the individual to be an "unfinished male."

The psychological analysis revealed the patient's emotional development and drives to be predominately masculine, thus correlating the personality studies with the hormonal findings and the physical development.

The subsequent treatment consisted of surgical procedures (laparotomy and later a plastic operation to free the bound down penis) and intensive testosterone therapy. This plan of treatment proved to be justified as the testosterone therapy enhanced the development of male secondary sex characteristics, and resulted in a marked improvement in the patient's general health, energy and endurance. Psychologically there was a great improvement in his outlook on life, and the patient is now stimulated to further study and achievement.

As long as therapy is continued, the patient's physical and psychological status continues to improve. Interruption of therapy for any appreciable length of time results in diminution of energy, endurance, and mental alertness. It is, therefore, evident that testosterone therapy must be continued indefinitely in order to maintain the results achieved.

ACKNOWLEDGMENT

The author is greatly indebted to Dr. Bernard Goldberg for his cooperation in carrying out extensive psychological studies on the patient and for the guidance given in the management of the case.

I wish to thank Dr. Konrad Dobriner of the Memorial Hospital, New York for his courtesy in carrying out the determination of the 17-ketosteroids and Dr. R. Kurzrok for his courtesy in carrying out the urinary bio-assays; Dr. B. L. Brent and Dr. Leo Pirk of Roche-Organon, Inc. for

supplying the chorionic gonadotropin (Pregnyl); Mr. C. E. Munson of Ciba Pharmaceutical Co. for supplying the methyl testosterone linguets (Metandren Linguets) and Dr. W. H. Stoner of Schering Corporation for the testosterone pellets (Oreton-F pellets) and testosterone ampules (Oreton).

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Letter to the Editor

To the Editor:

ESTRADIOL MONOBENZOATE (M.B.) CRYSTALS*

THE results in 22 patients with endocrine disorders which were treated in the Gynecological Clinic, Basle, between May 1946 and February 1947 are described.

Treatment with estradiol monobenzoate (M.B.) crystals was started on 19 women, ranging in age from 40 to 56 years; and on 3 women, 24, 33 and 38 years of age respectively. Various medicaments had already been tried therapeutically on most of the women, without success.

As compared with earlier trials, all the women, with one exception, tolerated the injections without any side effects such as pain or swelling at the site of the wound. We believe the tolerance is solely due to the technique chosen by us for the administration of the crystals.

After disinfecting the outside right or left buttock with alcohol and iodine, a deep local anesthetic (about 5 cc. novocaine with 1 drop of adrenalin 1:1000) is injected with a long needle. In order to avoid irregular wound surfaces, a passage is made after the anesthetic, by means of a puncture needle with a stilette. The stilette is then removed, and the syringe containing the crystals is attached to the cannula. After the injection, the stilette is once more pushed into the cannula so that the crystals which are still clinging to the sides of the cannula, are propelled into the crystal depot. By this method, the wound surface remains very small and the greater part of the crystals reaches the desired site of administration.

With regard to the patients treated, there were 2 cases of preclimacteric ovarian deficiency; 4 women were in the menopause; 7 were castrated by roentgen ray and 9 were castrated surgically. The last group of castrates is particularly important, because it is just these cases that are refractory to any kind of therapy. The tabular survey (Table 1) shows the dosage and effect of the estradiol monobenzoate crystals in detail. All the patients tolerated the crystal administration well, with one exception. In this instance 25 mg. of 0.3–0.4 mm. crystals were injected and prolonged painful sensations and swelling occurred at the site of the wound.

Although duration of effect was longest with a dose of 25 mg. in 0.2 to 0.4 mm. size, no further tests as to side effects were made with this dose and size of crystals.

Estradiol crystals in doses of from 10 and 25 mg. (0.2 to 0.3 mm. size)

* From the Department of Gynecology, Basle University, Basle, Switzerland.

TABLE 1. SUMMARY OF CASES WITH DOSAGE AND EFFECT OF ESTRADIOL MONOBENZOATE CRYSTALS MEASURING FROM 0.1 TO 0.4 MM.

Name	Age	Diagnosis	Dosage		Duration of Effect
	yrs.		mg.	mm.	
N.P.	42	Preclimacteric ovarian deficiency.	10	0.1–0.2	No effect
			25	0.1–0.2	14 days
			10	0.2–0.3	up to 3 weeks
			10	0.3–0.4	up to 2 months
F.C.	46	Preclimacteric ovarian deficiency.	10	0.2–0.3	14 days
M.C.	55	Menopause: ovarian deficiency.	10	0.2–0.3	about 6 weeks
S.G.	52	Menopause: ovarian deficiency.	10	0.1–0.2	5 days
			10	0.2–0.3	about 12 days
H.G.	54	Menopause: ovarian deficiency.	10	0.1–0.2	about 10 days
			10	0.3–0.4	3 months
			10	0.2–0.3	14 days
			25	0.2–0.3	about 4 months
G.B.	51	Menopause: pruritus vulvae	10	0.2–0.3	about 4 weeks
F.E.	52	Roentgen ray castrate	10	0.2–0.3	3 weeks
			10	0.3–0.4	5 weeks
			10	0.3–0.4	6 weeks
S.G.	47	Roentgen ray castrate	10	0.2–0.3	4 weeks
S.M.	56	Roentgen ray castrate	10	0.1–0.2	2 weeks
W.D.	47	Roentgen ray castrate	10	0.1–0.2	19 days
			25	0.1–0.2	7 weeks
			25	0.2–0.3	over 3 months
M.I.	48	Roentgen ray castrate	10	0.2–0.3	3 weeks
			10	0.2–0.3	over 4 months
R.G.	50	Roentgen ray castrate	10	0.1–0.2	about 10 days
			25	0.1–0.2	about 2 weeks
			10	0.2–0.3	5 weeks
			25	0.2–0.3	over 3 months
M.F.	24	Surgical castrate	10	0.2–0.3	3 weeks
			10	0.3–0.4	6 months

TABLE 1—(*continued*)

Name	Age	Diagnosis	Dosage		Duration of Effect
	yrs.		mg.	mm.	
B.G.	53	Surgical castrate	10	0.2–0.3	10 days
			25	0.2–0.3	up to 3 weeks
L.G.	42	Surgical castrate	10	0.1–0.2	*No effect*
			25	0.1–0.2	*No effect*
			10	0.3–0.4	about 5 weeks
H.J.	51	Surgical castrate	25	0.2–0.3	4 weeks
L.G.	50	Surgical castrate	10	0.2–0.3	2 months
			10	0.2–0.3	3 months
			10*	0.3–0.4	3½ months
			25*	0.3–0.4	about 6 months
K.R.	50	Surgical castrate	10	0.1–0.2	14 days
			25	0.1–0.2	about 10 weeks
J.S.	33	Surgical castrate	10	0.1–0.2	12 days
			25	0.1–0.2	3 weeks
			10	0.2–0.3	5 weeks
J.Z.	46	Surgical castrate	10	0.3–0.4	2 months
W.K.	38	Surgical castrate	10	0.2–0.3	weeks

* Pain for 2 weeks at the site of injection.

TABLE 2. SUMMARY OF DURATION OF EFFECT OF ESTRADIOL MONOBENZOATE CRYSTALS MEASURING FROM 0.1 TO 0.4 MM.

Number of Cases	Dose	Size of Crystal	Duration of Effect in Days			Remarks
			Min.	Max.	Average	
	mg.	mm.				
8	10	0.1–0.2	2	14	8.8	2 failures
6	25	0.1–0.2	5	70	28.8	1 failure
16	10	0.2–0.3	10	90	30.9	no failures
7	25	0.2–0.3	21	120	71.4	no failures
9	10	0.3–0.4	35	180	72.1	no failures
1	25	0.3–0.4	—	—	180	no failures

are best suited for treatment (See Table 2). Small crystals measuring from 0.1 to 0.2 mm. do not produce an adequate effect. The large crystals of 0.3 to 0.4 mm. size necessitate a larger and thicker cannula. The 0.2 to 0.3 mm. size in doses of from 10 to 25 mg. represent the most practical form of administration.

The following advantages have resulted from the use of crystals for injections in estrogen therapy:

1) There are fewer injections as compared with the usual method.

2) In contrast to tablet medication, there is the guarantee that the hormone is administered without an overdosage.

3) It is a simpler method than implantation and the dose is better controlled.

It can be concluded that estradiol administered in the form of crystals is an important addition to our therapeutic equipment.

Charles A. Joel M.D., Ph.D.
Lugano-Cassarate
Villa Bellosguardos
Switzerland
October 20, 1947

Book Review

RIDDLE, OSCAR. *Endocrines and Constitution in Doves and Pigeons.* Washington, D.C., Carnegie Institution of Washington, Department of Genetics (Pub. 572). 1947.

This study deals primarily with the outcome of an attempt to isolate strains or races of ring doves with diverse endocrine-associated traits through choice of 24 pairs of doves (from more than 200 pairs) whose reproductive performance and ancestry were known. Selection sometimes continued for two generations; inbreeding was sustained. Outcrosses provided many hybrid types and also further tests of the heritability of traits. Collection of data from all types was continued during 24 years. Of secondary importance are related results obtained from 12 races of domestic pigeons and from 15 derived hybrid types; from them was developed a race of hermaphrodite-producing pigeons. Exceptionally abundant data were obtained on the role of sex, hybridity, and race in heat production.

The primary tests with doves successfully established races with the following traits: With or without sex difference in body weight; small or large thyroids; long or short intestines; high or low response to the hormone prolactin; larger or smaller pituitary glands; early or late sexual maturity in females; larger or smaller eggs; larger or smaller juvenile testes; high or low rate of heat production. Widespread genetic inequalities of individuals and races were thus demonstrated in this material. Most of these results are theoretically applicable to man, and thus provide cogent evidence for biological inequality of human individuals, types, and races.

Abstracts of

CURRENT ENDOCRINE LITERATURE

Editor; D. A. McGINTY. *Collaborators:* A. R. ABARBANEL, F. N. ANDREWS, B. L. BAKER, F. A. DE LA BALZE, ISRAEL BRAM, R. A. CLEGHORN, RUCKER CLEVELAND, C. D. DAVIS, ANNA FORBES, M. B. GORDON, H. S. GUTERMAN, M. M. HOFFMAN, R. G. HOSKINS, C. D. KOCHAKIAN, H. S. KUPPERMAN, H. L. MASON, JANET W. MCARTHUR, THOMAS H. MCGAVACK, A. E. MEYER, K. E. PASCHKIS, A. B. PINTO, J. R. REFORZOMEMBRIVES, E. C. REIFENSTEIN, JR., G. G. RUDOLPH, L. T. SAMUELS.

THE OVARIES

GREEN-ARMYTAGE, V. B. SILBERSTEIN, F., AND WACHTEL, G. E.: The influence of semen on the female reproductive organs, J. Obst. and Gynec. Brit. Emp. 54 (3): 324–339, 1947.

The authors found injections of semen to have a follicle-stimulating effect in rats, mice and rabbits. These were given intraperitoneally or intramuscularly and also intravenously in rabbits. The effects were obtained with human as well as with homologous semen.—*R.A.C.*

SNAITH, L., AND WILLIAMSON, M.: Pregnancy after the menopause, J. Obst. and Gynec. Brit. Emp. 54 (4): 496–498, 1947.

A 45-year old multipara, whose menstrual periods had ceased 3 years before, became pregnant and was delivered of a normal child. Although she had nursed her previous children, this time lactation failed completely. The literature of similar cases is reviewed. —*R.A.C.*

OBERMER, E.: Calcium and phosphorus metabolism in pregnancy (A survey under war and post-war conditions). First communication on the temperamental and emotional factor, J. Obst. and Gynec. Brit. Emp. 54 (4): 432–442, 1947.

This is the third of a series of communications and summarizes conclusions from all three. A daily minimal intake consistent with safety is held to be 1.5 Gm. for phosphorus and for calcium 2 Gm. to the 8th month and 2.5 Gm. thereafter. Most adequate diets supply the above amount of phosphorus but not of calcium. In Britain a supplement of Calciferol is essential; 6,000 units during the first 6 months and 12,000 in the last 3 for placid women. For the highly strung, larger quantities are said to be necessary in virtue of the influence of the endocrines and autonomic nervous system on metabolic function. —*R.A.C.*

KARNAKY, K. J.: The effect of oestrogen (Stilboestrol) on the formed elements of the blood in women, J. Obst. and Gynec. Brit. Emp. 54 (3): 366–368, 1947.

Stilboestrol had no apparent effect on platelet count, haemoglobin value or total

white cell count in 163 women receiving this drug in therapeutic doses over periods ranging from 1 week to a year. No purpura or other bleeding tendency was noticed.—*R.A.C.*

BIGGS, ROSEMARY AND ROSE, ELIZABETH: The familial incidence of adrenal hypertrophy and female pseudohermaphroditism, J. Obst. and Gynec. Brit. Emp. **54** (3): 369–374, 1947.

Case records are given of two female hermaphrodites born successively to normal parents who already had one normal female child. Death occurred in one at 6 weeks; in the other at 4 weeks of age, with symptoms reminiscent of Addisonian crises. At autopsy both had adrenals twice the normal weight for that age. The reports from the literature dealing with the familial incidence of female hermaphroditism and the occurrence of males in the same families showing precocious puberty and male pseudohermaphroditism are detailed.—*R.A.C.*

THE ADRENALS

WILHELM, S. F.: Roentgenographic delineation of the adrenal glands with the aid of laminography, Br. J. Urol. **19**:85, 1947.

The laminographic technique was found superior to simple films for visualizing the adrenal glands after perirenal insufflation with air or oxygen. Seventy cases were studied without deaths or serious sequelae.—*R.A.C.*

ANNOUNCEMENT OF THE 1948 MEETING OF THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS

The Thirtieth Annual Meeting of the Association for the Study of Internal Secretions will be held in the Palmer House, Chicago, Illinois, June 18 and 19, 1948.

The scientific sessions will be held in the Red Lacquer Room and registration will be on the fourth floor just outside the Red Lacquer Room. The Annual Dinner will be held in the same room on Friday, June 18th at 7 P. M. and will be preceded by a cocktail party, the location of which will be announced later.

All members of the Association who plan to attend the Thirtieth Meeting are urged to make their reservations at once with the Palmer House, stating the time of arrival and how long they plan to remain in Chicago.

Nominations for awards and fellowships of the Association must be in the Secretary-Treasurer's office by March 15, 1948.

Those wishing to present papers should send title and four copies of a comprehensive abstract of not more than 200 words and suitable for publication in the program to Dr. C. N. H. Long, 333 Cedar Street, New Haven, Conn., not later than March 15, 1948.

Announcement of Awards and Fellowship of the Association

Nominations for Awards

Three awards for meritorious work in endocrinology will be given at the next annual meeting of the Association. A special committee of five members of the Association chooses the recipients of these Awards, subject to ratification by the Council, and each member of the Association has the privilege of making one nomination for each award.

Nominations for the Awards should be made on special application forms which may be obtained from the Secretary, Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City 3, Oklahoma. All nominations, accompanied by a statement of the importance of the nominee's contributions to endocrinology and a bibliography of his most important papers with reprints if possible, should be sent to Dr. Turner's office not later than March 15, 1948.

THE E. R. SQUIBB AND SONS AWARD

The E. R. Squibb and Sons Award of $1,000.00 was established in 1939. It was given in 1940 to Dr. George W. Corner; in 1941 to Dr. Philip E. Smith; in 1942 to Dr. Fred C. Koch; in 1944 to Dr. Edward A. Doisy; in 1945 to Dr. E. C. Kendall; in 1946 to Dr. Carl G. Hartman; in 1947 to Drs. Carl F. and Gerty T. Cori. No award was made in 1943. No age or special limitation is stipulated by the donor of the award.

THE CIBA AWARD

The Ciba Award, established in 1942, is given in recognition of the meritorious accomplishment of an investigator, not over 35 years of age, in the field of clinical or pre-clinical endocrinology. In 1944 the Award was given to Dr. E. B. Astwood; in 1945 to Dr. Jane Anne Russell; in 1946 to Dr. Martin M. Hoffman and in 1947 to Dr. Choh Hao Li. The Award is for $1,200.00. If within two years of the date of the Award, the recipient chooses to use it to aid in working in a laboratory other than the one in which he normally is located, the Award will be increased to $1,800.00.

THE AYERST, McKENNA & HARRISON FELLOWSHIP

The first award of the Ayerst, McKenna & Harrison Fellowship was given to Dr. Samuel Dvoskin in 1947. The fellowship was founded in order to encourage investigation in the field of endocrinology rather than as an award

for work done. The amount of the fellowship is $2,500.00 annually. The nominee must possess the degree of Doctor of Philosophy or Doctor of Medicine or their equivalent. It is suggested that no restriction be placed on age, but that preference be given to applicants who have recently completed the requirements for their Ph. D. or M. D. degree. The nominee must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals; submit a program of proposed study; indicate one or more institutions where the proposed program will be carried out; submit statement of approval from the investigators with whom he proposes to conduct his research; serve full time if awarded a fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.

Postgraduate Course in Endocrinology

The Postgraduate Committee of THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS, under authority of its Council, announces a course of lectures and demonstrations in CLINICAL ENDOCRINOLOGY to be held in LOS ANGELES at the BILTMORE HOTEL, FEBRUARY 23 to 28, 1948, inclusive.

The faculty will consist of the most prominent investigators and clinical endocrinologists in the various branches of the medical sciences in the United States and Canada.

It is the intent of the Committee that this course be a practical one of interest and value to both the GENERAL PRACTITIONER AND THE SPECIALIST.

A fee of $100 will be charged for the entire course and the attendance will be limited to 100. Registration will be in the order of checks received and will close on February 1, 1948. Should there be an insufficient number of applicants to warrant the course, the registration fee will be immediately refunded in full.

Please make your application on your letterhead and forward, together with your check payable to THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS, to DR. E. KOST SHELTON, CHAIRMAN of the POSTGRADUATE COMMITTEE, 921 WESTWOOD BOULEVARD, LOS ANGELES 24, CALIFORNIA.

Since satisfactory hotel accommodations are still difficult to procure on short notice in Los Angeles, especially during the winter season, it is suggested that all applicants MAKE THEIR RESERVATIONS EARLY.

SOME LARGE HOTELS IN THE METROPOLITAN AREA OF LOS ANGELES:

Alexandria	Chapman Park	Lankershim
Ambassador	Gaylord	Mayflower
Biltmore	Hayward	Town House

AMERICAN ASSOCIATION FOR THE STUDY OF GOITER

VAN METER PRIZE AWARD

The American Association for the Study of Goiter again offers the Van Meter Prize Award of Three Hundred Dollars and two honorable mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The Award will be made at the annual meeting of the Association which will be held in Toronto, Canada, May 6th, 7th, 8th, 1948 providing essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations; should not exceed three thousand words in length; must be presented in English; and a typewritten double spaced copy sent to the corresponding secretary, Dr. T. C. Davison, 207 Doctors Building, Atlanta 3, Georgia not later than February 1st, 1948. The committee, who will review the manuscripts, is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for presentation of the Prize Award Essay by the author if it is possible for him to attend. The essay will be published in the annual Proceedings of the Association. This will not prevent its further publication, however, in any Journal selected by the author.

T. C. Davison,
Corresponding Secretary

AMERICAN COLLEGE OF PHYSICIANS RESEARCH FELLOWSHIPS IN MEDICINE 1948 AWARDS

The Board of Regents of the College, on the nomination of the Committee on Fellowships and Awards, awarded six Research Fellowships in Medicine for the year beginning July, 1948, at their meetings in Philadelphia on November 22 and 23, 1947. The awards were made to the following physicians.

Charles Gordon Campbell, M.D., C.M., Vancouver, B. C., Can. Dr. Campbell attended the University of British Columbia and is a graduate of the McGill University Faculty of Medicine, 1945. He interned in the Royal Victoria Hospital, Montreal, 1945–46, and has held appointments in the Vancouver General Hospital as Assistant Resident in Medicine, 1946–47, and as Fellow in Cardiology, 1947–48. Dr. Campbell will undertake studies of the basic physiology of certain cardiovascular problems in the Department of Physiology of McGill University, under the supervision of Professor Hebbel E. Hoff.

Frank Herbert Gardner, M.D., San Bernardino, Calif. Dr. Gardner attended Northwestern University (B. S., 1941; M.D., 1944). He served as intern and Assistant Resident in the San Francisco Hospital, 1944–46, and as Senior Assistant Resident in the University of California Hospital, 1946–47. Presently Fellow in Medicine in the Boston City Hospital, Dr. Gardner will conduct studies in the Thorndike Memorial Laboratory under the direction of William B. Castle, M.D., F.A.C.P., and Thomas H. Ham, M.D., F.A.C.P., of the mechanism and clinical application of the osmotic fragility test.

Samuel P. Martin, M.D., Durham, N. C. Dr. Martin's premedical and medical courses were taken at Washington University, St. Louis, where he received the M.D. degree in 1941. He interned in the Barnes Hospital, St. Louis, 1942–43, and served there also as Assistant Resident in Medicine, 1943–44. Dr. Martin served in the Army from 1944 until 1947. He is pres-

ently Resident in Medicine in the Duke University Hospital. With the aid of the Fellowship, Dr. Martin will undertake studies of bacterial metabolism in the Rockfeller Institute for Medical Research, New York, N. Y., under the direction of Dr. René J. Dubos.

Peritz Scheinberg, M.D., Miami, Fla. Now Assistant Resident in Medicine in the Duke University Hospital, Dr. Scheinberg attended Emory University, where he received the A.B. degree in 1941, and the M.D. degree in 1944. He subsequently served as intern and Assistant Resident in Medicine in the Grady Memorial Hospital, Atlanta, Ga. Dr. Scheinberg served in the U. S. Naval Reserve, 1945–46. He will conduct an investigation of cerebral circulation and peripheral vascular flow in normal and hypertensive persons in the Duke University Hospital under the direction of Eugene A. Stead, Jr., M.D., F.A.C.P.

Lutfu Lahut Uzman, M.D., Istanbul, Turkey. Dr. Uzman will conduct studies, now under way, with Dr. J. Folch-Pi, in the Department of Scientific Research of the McLean Hospital, Waverly Mass., on the isolation and characterization of brain proteins and their role in health, disease and senescence. Dr. Uzman received the B.S. degree from the University of Istanbul Faculty of Science in 1940. Following several years of study in the Medical School of that institution, he transferred to the Harvard Medical School and completed his work for the M.D. degree there in 1946. He interned in the Boston City Hospital, Neurology Service, 1946–47, and has since held appointment as Assistant in the McLean Hospital.

John Martin Weller, M.D., Ann Arbor, Mich. Dr. Weller completed his undergraduate studies at the University of Michigan in 1940 and received the M.D. degree from Harvard Medical School in 1943. He served as Medical House Officer, Peter Bent Brigham Hospital, Boston, from January to October, 1944; as Assistant Resident in Medicine in the Vanderbilt University Hospital, Nashville, Tenn., from October, 1944, to July, 1945. Since January of 1946, Dr. Weller has been Medical Resident in the Veterans Administration Hospital, Hines, Ill. Dr. Weller's appointment to the first Alfred Stengel Research Fellowship of the American College of Physicians will enable him to undertake, with Professor A. Baird Hastings in the Department of Biologic Chemistry of Harvard Medical School, studies concerning the ionic patterns of the intracellular fluids and their influence on enzymatic reactions; of acid-base balance in tissues other than skeletal muscle tissues.

NATIONAL RESEARCH COUNCIL GRANTS FOR RESEARCH IN ENDOCRINOLOGY

The Committee on Research in Endocrinology, National Research Council, wishes to announce that requests for grants-in-aid during the fiscal period from July 1, 1948, to June 30, 1949, will be received until February 29, 1948. Application blanks may be obtained by addressing the Secretary, Division of Medical Sciences, National Research Council, 2101 Constitution Avenue, Washington 25, D. C. In Addition to a statement of the problem and research plan or program, the Committee desires information regarding the proposed method of attack, the institutional support of the investigation and the uses to be made of the sum requested. No part of any grant may be used by the recipient institution for administrative expenses.

The Committee makes grants in aid of research in the general field of experimental and clinical endocrinology. However, applications for support of research in the problems of sex in the narrower sense cannot be given favorable consideration, and investigators seeking support in this field should direct their proposals to the Committee for Research in Problems of Sex of the National Research Council. The Committee on Research in Endocrinology, however, will continue to give consideration to the support of studies of the effect of sex hormones on non-sexual functions, e.g., on general metabolism and on the metabolism of steroid hormones.

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A FEMINIZING ADRENAL TUMOR CAUSING GYNECOMASTIA IN A BOY OF FIVE YEARS CONTRASTED WITH A VIRILIZING TUMOR IN A FIVE-YEAR-OLD GIRL

CLASSIFICATION OF SEVENTY CASES OF ADRENAL TUMOR IN CHILDREN ACCORDING TO THEIR HORMONAL MANIFESTATIONS AND A REVIEW OF ELEVEN CASES OF FEMINIZING ADRENAL TUMOR IN ADULTS*

LAWSON WILKINS, M.D.

From the Department of Pediatrics, Johns Hopkins University School of Medicine, and the Harriet Lane Home of the Johns Hopkins Hospital, Baltimore, Maryland.

TWO children, a boy and a girl, each 5 years old, with marked changes toward the adult characteristics of the opposite sex, were studied at about the same time in the Endocrine Clinic of the Harriet Lane Home. In each case an encapsulated adenoma of the right adrenal was successfully removed. The cases are of interest as examples of the difference in the hormonal effects of adrenal tumors and because hitherto no instance of gynecomastia due to adrenal tumor has been reported in a boy before puberty.

CASE REPORTS

Case 1. Donald G. (H.L.H. A 31153) came to our attention July, 1943, at the age of 4 years and 8 months because of large, well developed breasts. During the neonatal period hyperplasia of the breasts so common among newborns was not noted, but at the

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age of 6 months the breasts became enlarged and attained their present state of development by the age of 1 or 2 years. There had been no tenderness and no secretion. Except for the breasts, the boy's growth, development and general health had been normal in every way. He behaved like a normal boy and showed no feminine traits of personality.

At the time he was examined, he was only 1 inch taller than the average and his skeletal proportions were entirely normal. However, his epiphysial development was that of a 10-year old boy, and his dental development was slightly advanced (Fig. 1).

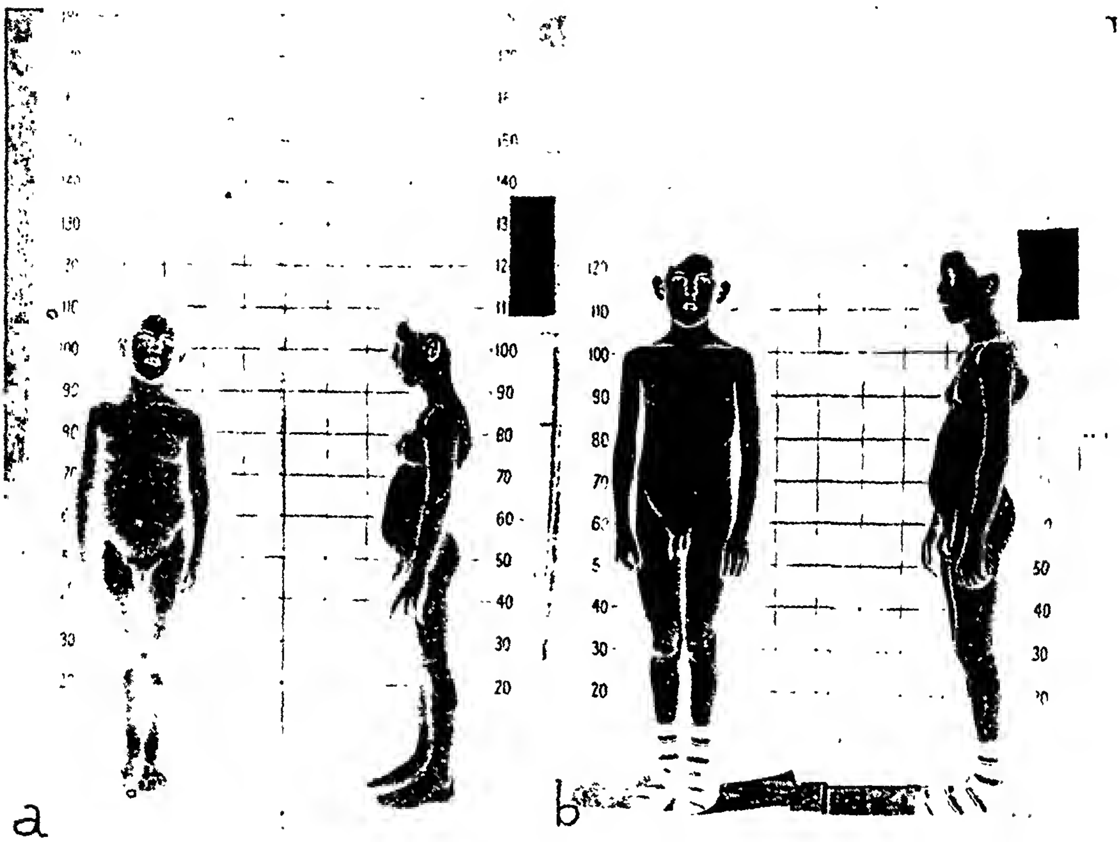

FIG. 1a. *Case 1:* D.G. age 5 years. Before operation. Note the well developed breasts and nipples and normal male genitalia.
FIG. 1b. *Case 1:* D.G. age $8\frac{1}{4}$ years. Three years after operation. Breasts much smaller. No signs of puberty.

The breasts were conical and measured 9 cm. in diameter at the base. The central area of 5 cm. diameter felt like normal glandular structure. The areolae were well developed but did not show much pigment. The nipples were small. There were well marked pectoral and circumareolar veins. No secretion could be expressed. The penis was normal for the patient's age and the testes were of normal size and consistency, measuring 2×1.2 cm. The prostate was definitely enlarged for his age, about one-third the adult size and was of unusually firm fibrous consistency. There was no pubic hair. The skin was normal and showed no unusual pigmentation or seborrhea.

The general examination was essentially negative. Palpation of the abdomen revealed no masses. X-rays of the bones, skull and lungs showed no tumor metastases. Intravenous pyelograms showed a double renal pelvis and a bifid ureter on the right side.

Above the right kidney and apparently not connected with it, there was a calcified area 1 cm. in diameter visible on both the flat X-ray plate and the intravenous pyelogram. Urine examination and P.S.P. showed no abnormality. The intradermal tuberculin test was negative. Blood counts were normal. Chemical examination of the serum showed the concentration of N.P.N. to be 19 mg. per cent, calcium 11.9 mg. per cent, phospho-

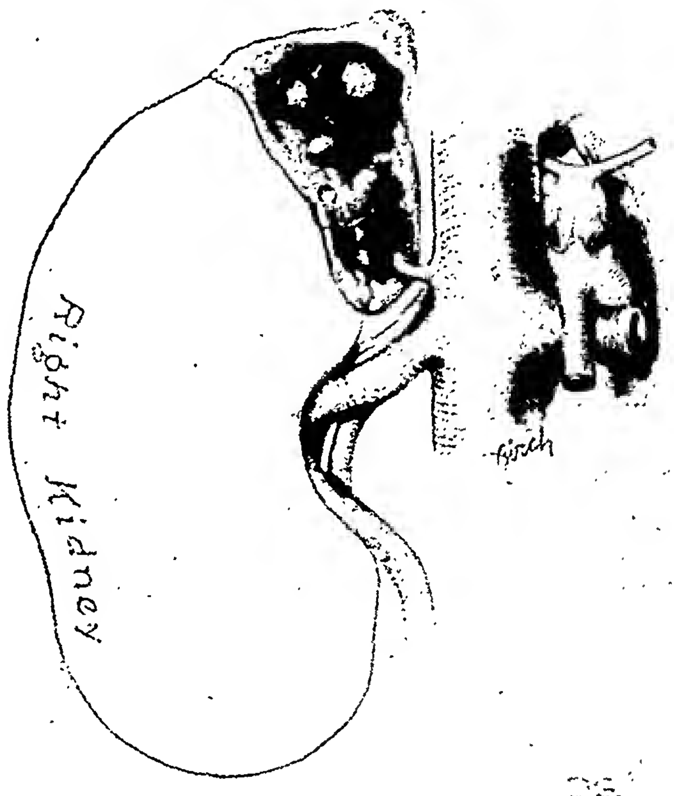

FIG. 2. *Case 1:* Drawing of adrenal tumor causing gynecomastia.

rus 5.3 mg. per cent, phosphatase 8.7 Bodansky units, chloride 101 millequivalents, and cholesterol 132 mg. per cent. Glucose tolerance and insulin sensitivity tests were negative. The Kepler-Powers diuresis test for adrenal function revealed no abnormality. The 17-ketosteroid excretion was 4.1 mg. per 24 hours. Assay of urinary estrogen (Dr. Delfs) showed an excretion of 5 rat units per 24 hours which is only slightly greater than normal for even an adult male. Pregnandiol was not detected in the urine. An assay of F.S.H. was negative in a titer of 6.5 mouse units per 24 hours.

Because of the occurrence of gynecomastia at this early period of life, and because of the excretion of estrin in the urine, it was believed the boy probably had a tumor producing an estrogenic substance. The possibility of a testicular tumor, an embryonic tumor somewhere in the abdominal cavity or a tumor of the adrenal was considered.

The only suggestion as to its location was the calcified shadow in the right suprarenal region.

It was felt that a transperitoneal incision which would permit exploration of the abdomen as well as approach to the adrenal was indicated. This was performed by Dr. Alfred Blalock and Dr. Hugh Jewett November 16, 1943. No abnormality was found in the pelvis or abdominal cavity. Exposure of the right adrenal immediately revealed a tumor of dark brown color about the size of an olive (Fig. 2). On its anterior surface there appeared to be two nodules but on its posterior surface it was apparently a single

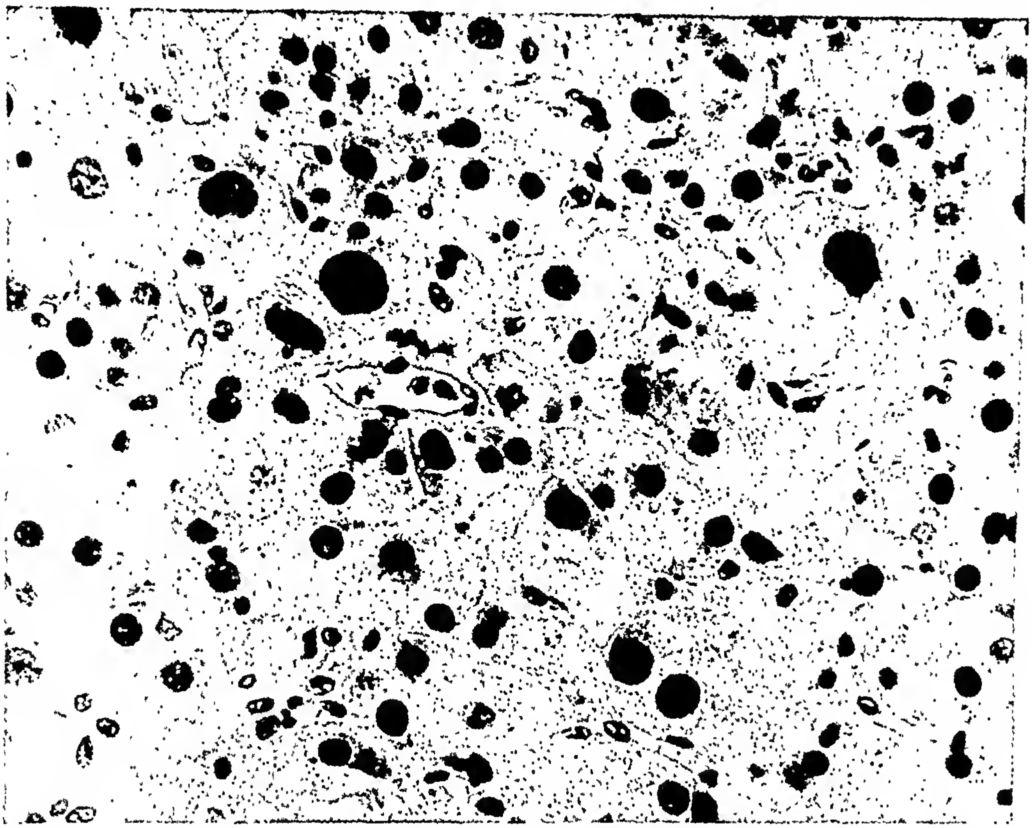

FIG. 3. *Case 1:* Microphotograph (×400) of adrenal tumor causing gynecomastia.

mass. The tumor was removed together with some adrenal tissue, leaving only a small portion of the upper pole of the adrenal. The left adrenal was carefully examined and appeared normal.

Pathological Examination by Dr. Sam Blackman: "The specimen removed consisted of adrenal gland and tumor measuring 3×2 cm. Occupying a large proportion of this and arising slightly closer to one end than the other, there is a trilobular firm tumor which on one side reveals two nodules projecting from the surface of the adrenal and on the other side, a single larger lobule. One lobule is calcified and is cut with difficulty. The rest of the tumor is soft and brown in color.

Microscopic Examination: (Fig. 3). The tumor is separated from the normal adrenal by a fibrous capsule. There are calcified areas in the capsule and scattered calcified areas in the tumor itself. The section fixed in Vandegrift's solution shows very large cells although some are only moderately large. The cells vary in shape a good deal. They have

deeply eosinophilic coarse granular cytoplasm. Some of the cytoplasm has dropped out. The outline of every cell is sharp, so that it appears almost as if there were a distinct membrane around each cell. The nuclei are mostly round and vary greatly in size. Some are huge, hyperchromatic nuclei; many contain nucleoli. Tissue fixed in Zenker's solution shows the same large cells of irregular shape and the same variable nuclei, many of which are enormous and extremely hyperchromatic. There are no mitotic figures. In

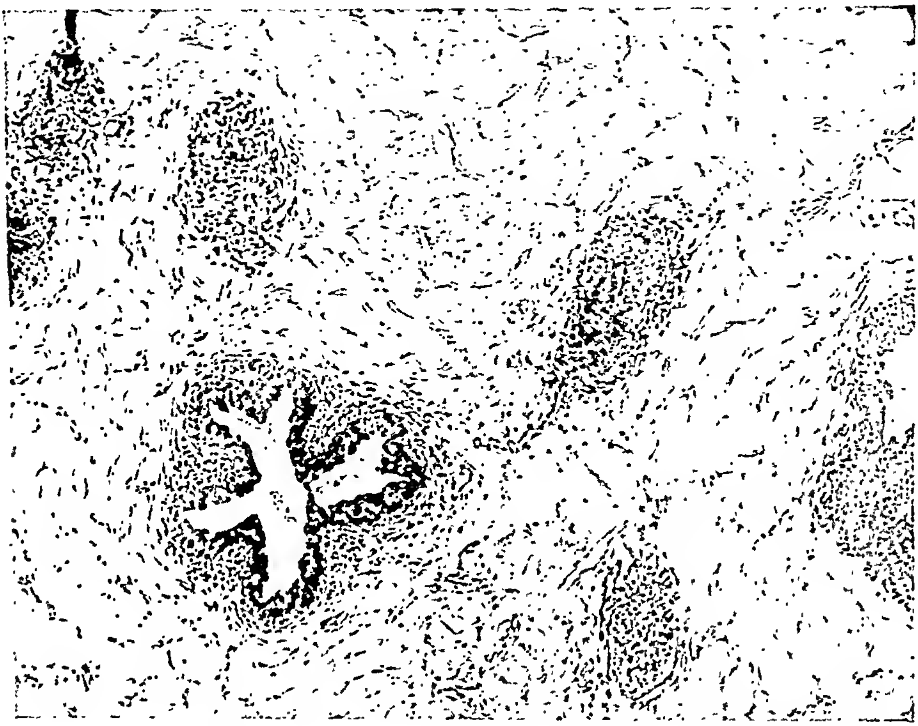

FIG. 4. *Case 1:* Microphotograph (×90) of breast biopsy. Relatively slight hyperplasia of ducts but marked proliferation of periductal fibrous stroma.

the Zenker-fixed tissue, however, the outlines of the cells are less distinct, and the cytoplasm has not dropped out. Moreover, the appearance of a cell membrane is not present in this tissue at all. Many of the cells in the Zenker-fixed tissue contain relatively large droplets of yellowish brown material which is highly refractive and accounts for the brownish color of the gross specimen. These pigmented drops were evidently dissolved out in the Vandegrift's fixed tissue, probably by the acetone. They are entirely different from the minute, more brown granules of pigment that are found in tumors and hyperplasias of the adrenogenic zone of the adrenal cortex (1). Sections fixed in Zenker's fluid and stained with iron-hematoxylin show hematoxylin-stained granules and droplets in many of the cells, such as are found in the prenatal zone of the adrenal cortex. These did not form complete circles in the cells like the pigment granules and their preceding mitochondria which occur in the reticular zone cells of the adrenal cortex. With ponceau-fuchsin stain the protoplasm of the cells appears granular and stains deeply red. The cells

of this tumor are identical with microscopical cell masses which we have found in the prenatal zone of the adrenal cortex of infants at autopsy.

Biopsy of breast (Fig. 4) shows some hyperplasia of the duct epithelium and marked periductal hyperplasia of the connective tissue. The connective tissue appears old and hyaline and contains few nuclei. It is so widespread that it no longer shows any particular periductal arrangement, as is seen in early cases of gynecomastia. It is evident that the main mass of the enlarged breast consists of this hyaline connective tissue."

The boy made an uneventful recovery. In the 4 years since operation he has been examined repeatedly in the Endocrine Clinic. His health has been good and his growth and development normal. The breasts have decreased slowly but progressively in size. They are still quite noticeable but measure only 3 to 4 cm. in diameter and are more soft and flabby. In June, 1947, at the age of 8 years and 9 months, he was 50.3 inches tall, which is within average range for this age. The bone age was about 9½ years. According to Dr. Jewett, the prostate had decreased somewhat in size but was still enlarged. There was almost a stony induration of the left lobe, whereas the right was of normal consistency. No calcification was demonstrated by X-ray.

Case 2. Jane C. (H.L.H. A 33040) was 5½ years old when she was first seen by us. The mother and the attending physician were certain that her genitalia were entirely normal at birth. At the age of 5 years, in May, 1943, the mother noticed that there was slight enlargement of the clitoris which grew rapidly from then on. About September, pubic hair began to appear and grew rapidly thereafter. Her voice became deeper, her features coarser, and her skin became oily and showed a little acne. Within a few months she grew 3 inches and gained 10 lbs., becoming more muscular.

In November, 1943, at the age of 5½ years her height was 44.6 inches which was 1½ inches taller than the average for her age (Fig. 5). The epiphysial development was that of 7 years. The musculature was well developed. Her features were a little more mature than normal for her age. There were seborrhea and slight acne but no abnormal pigmentation of the skin. There was a pronounced growth of coarse hair over the pubis and in the perianal region. There was some dark down on the upper lip but no axillary hair. The clitoris measured 3 cm. × 1 cm. The vulval configuration was entirely normal. The labia majora were somewhat hypertrophied and pigmented, while the labia minora were undeveloped and of preadolescent type. The meatus of the urethra was situated in the usual position near the base of the clitoris, and the vaginal orifice admitted a finger. In this respect she differed from female pseudohermaphrodites in whom the occurrence of androgenic hyperplasia of the adrenals in early embryonic life brings about persistence of the urogenital sinus. On rectal examination, Dr. TeLinde felt a small uterus and a small ovary on each side. No tumor was felt in the pelvis nor could any mass be felt in the abdomen or kidney region. Intravenous pyelograms were negative. Blood counts, blood chemical studies, glucose tolerance, insulin sensitivity tests and a water excretion test for adrenal function were all negative. The 17-ketosteroid excretion varied between 19 and 22 mg. per day, of which less than 1 per cent was the beta fraction.

Embryonic hyperplasia of the adrenal could be excluded in this case because of the normal development of the vagina and urethra. The sudden and rapidly progressive onset of virilization at this age suggested that the child had a virilizing tumor of the adrenal gland or of some aberrant adrenal tissue. On December 31, 1943, Dr. Richard W. TeLinde and Dr. Alfred Blalock performed a transperitoneal exploration through which they were able to inspect the pelvis as well as the abdomen. Normal uterus, tubes and ovaries of average size for her age were found. Exposure of the right adrenal showed a tumor of dark brown color the size of a hen's egg attached to the lower pole of the

gland by a comparatively narrow pedicle (Fig. 6). It was removed leaving the upper half of the gland. The left adrenal appeared entirely normal.

Pathological Examination: "The adrenal tumor measures 4 ×3 cm. There is a capsule about 0.5 cm. thick. The lower two-thirds is rather firm and lobulated. The proximal third is cystic in consistency. At one point the capsule has been ruptured and protruding

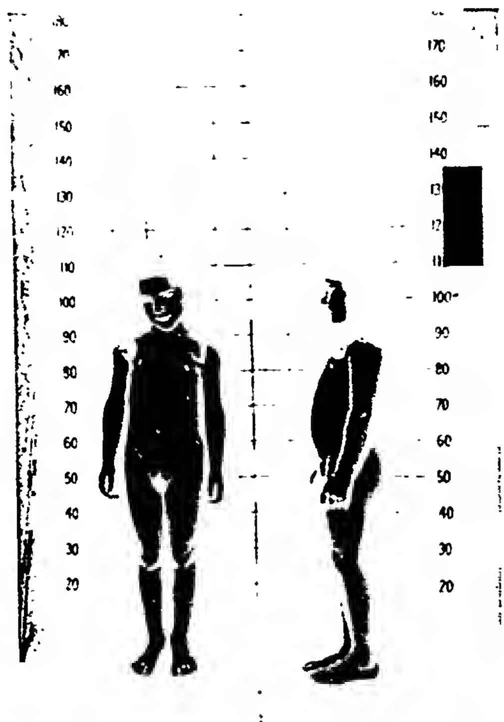

FIG. 5. *Case 2:* J.C. age 5½ years. Marked development of pubic hair. The greatly hypertrophied clitoris is not visible in the photograph. The vagina and urethra were normal.

through the opening is some very friable adrenal tissue with the typical brownish color. The tumor was attached by a pedicle about 1 cm. in diameter to the lower pole of the adrenal gland, which was not removed. The blood supply through this pedicle enters a small area of typical adrenal medulla.

Microscopic Examination: (Fig. 7) Sections show a well-encapsulated tumor composed of an irregular growth of cells having a granular pink-staining cytoplasm and containing nuclei which range in size from about 7 to 30 microns. The small nuclei have a dark pyknotic stain somewhat like lymphocytes. The larger ones have a reticulum and a very dark blue-staining central nucleolus. There are no mitotic figures, but there are a number of other pyknotic cells. There are scattered areas of soft calcification from 3 to 40 microns in diameter. In one of the sections there is a small amount of adrenal tissue outside the capsule. This is composed of strands of the usual small cells. Except

for the intact capsule and the absence of mitotic figures, the great disparity of size in the cell structure and of the staining qualities of the nuclei suggest a malignancy."

Following operation the child suffered no shock and recovered rapidly. The pubic hair which had been shaven grew rapidly again for about 3 weeks but was more scant than before operation. Her seborrhea and acne decreased. The 17-ketosteroid excretion which was from 19 to 22 mg. before operation decreased to 2.8 mg. the third day after operation, and to 1.4 mg. the eighth day. The patient was seen again in April, 1944. She had grown

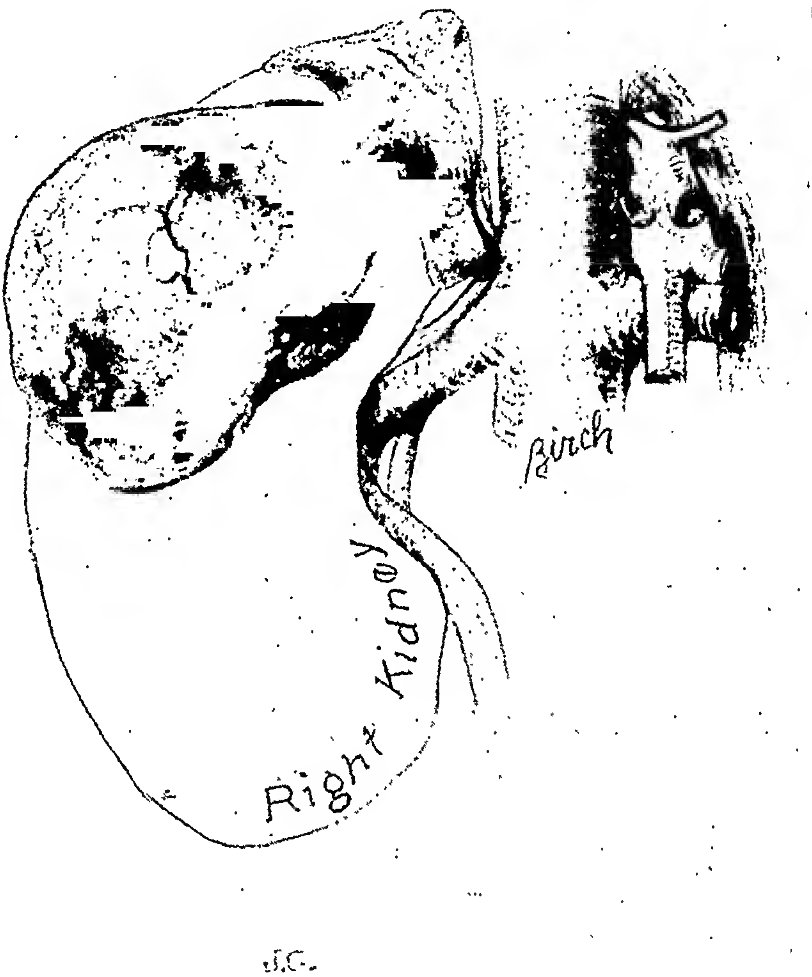

FIG. 6. *Case 2:* Drawing of adrenal tumor causing virilization.

2.2 inches since December, 1943. It was thought that the pubic hair was a little less dense than before operation and the clitoris was a little smaller. The excretion of 17-ketosteroids was 2.1 mg. per day. When she was seen again in December, 1944, the patient's height was 49.6 inches, representing a growth of 5 inches during the preceding year. The bone age, which was 7 the previous year, was now 11 years. The acne was more marked. Pubic hair was more abundant, a few hairs had appeared in the axillae and there was a little dark hair on the upper lip. Her voice was slightly deeper than previously. There was no change in the clitoris. There was no development of the breasts. The

vaginal smear indicated no estrinization. The 17-ketosteroids were 94 mg. per day December 27, and 126 mg. per day, December 28. X-rays of the chest and bones showed no metastasis. Intravenous pyelograms were negative. It was believed that the tumor had recurred or metastasized, and another abdominal exploration was undertaken by Dr. TeLinde on January 6, 1945. His note was as follows:

"On the lateral border of the ascending colon, about 2 inches from the tip of the caecum, there was a yellowish pedunculated tumor about the size of a golf ball. It had

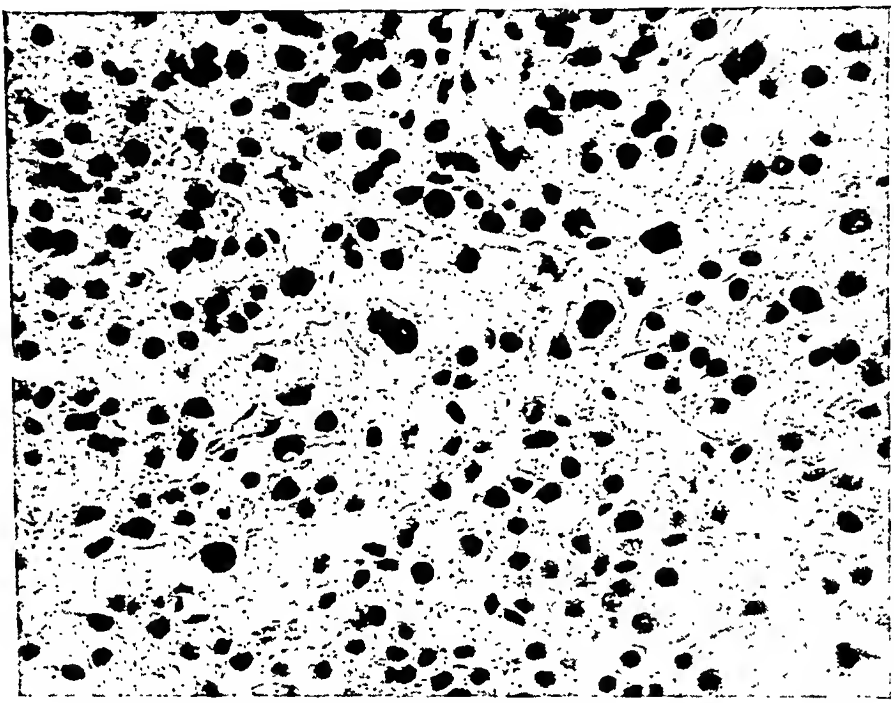

FIG. 7. *Case 2:* Microphotograph (×400) of adrenal tumor causing virilization.

the smooth appearance of the circumscribed tumor which was removed from the adrenal last year. At the hepatic flexure of the colon there was another similar tumor which was slightly larger. This tumor seemed quite free and very well encapsulated beneath the serosa of the colon. On the under surface of the right lobe of the liver to the right of the gall bladder a third tumor was present, which had the general appearance of the others but was more irregular in shape and blended with the liver substance. Above the upper pole of the right kidney in the region of the adrenal there was a small nodule about the size of a pea which probably represented a recurrence of the original tumor but there was no other evidence of recurrence in the adrenal region. The two large tumors attached to the colon were readily removed. The metastasis attached to the liver had apparently invaded that organ and it was impossible to remove it completely. The small tumor in the adrenal region was not removed."

Pathological Report: "The several sections of the three tumors show an identical microscopic picture. There are large sheets of the cells, the nuclei of which vary tre-

mendously in size and staining reaction. Mitotic figures are extremely rare. There is an abundant amount of pink cytoplasm and practically no connective tissue stroma. The tumor is well supplied with blood but in spite of this there are a few areas of necrosis and in these regions there is a mild infiltration with inflammatory cells. In the section taken from the liver there is invasion and destruction of the liver cells."

Following this operation there was a temporary decrease of the 17-ketosteroid excretion which then increased again as shown by the following:

17-Ketosteroids per day

12- 3-43	22.0 mg.
12- 4-43	19.0 mg.
12- 5-43	21.0 mg.
First operation 12-31-43	
1- 3-44	2.8 mg.
1- 5-44	2.5 mg.
1- 6-44	2.4 mg.
1- 7-44	0.9 mg.
1- 8-44	1.4 mg.
1- 9-44	1.5 mg.
1-12-44	1.7 mg.
4- 8-44	2.1 mg.
12-27-44	94.0 mg.
12-28-44	126.0 mg.
Second operation 1-4-45	
1-10-45	16.0 mg.
1-14-45	18.0 mg.
1-21-45	35.0 mg.
2- 1-45	38.0 mg.
3- 6-45	70.0 mg.
4-15-45	124.0 mg.

Following the second operation she returned to her home in another city. She is said to have remained strong and healthy until 2 weeks before her death in December, 1945. No details of her last illness are available.

DISCUSSION OF CASES

The hormonal effects of the two adrenal tumors were quite different. The girl showed enlargement of the phallus, precocious growth of sexual hair, seborrhea, deepened voice, somatic and muscular growth and accelerated epiphysial development. All of these symptoms can be attributed to excessive secretion of androgen. There were no signs of excessive cortin activity such as obesity, striae, plethora, hypertension or diabetic sugar curve. The lack of breast development and the absence of adolescent changes in the labia minora or vaginal epithelium indicated that estrogen was not being produced in as great amounts as in the adolescent period, or else that its effects were suppressed by excessive androgen. In the case

of the boy there were no evidences of excessive production of either androgen or cortin, and the excretion of 17-ketosteroids was low, not exceeding 4 mg. per day. The enlargement of the breasts suggested that estrin was being elaborated in excess. However, the breasts differed from normal female mammae in containing a large amount of connective stroma with little proliferation of ducts. Breast development cannot be considered proof of estrogenic activity because experimentally mammogenic effects may be produced by testosterone, chorionic gonadotropin or desoxycorticosterone. The cause of the breast enlargement in adolescent males is uncertain. In the syndrome described by Klinefelter et al. (2) and by Heller and Nelson (3), Albright has postulated that the gynecomastia is due to lack of "inhibin" secreted by the seminiferous tubules while androgen continues to be secreted by the interstitial cells. The fact that our patient had an enlarged prostate which seemed to be firm and fibrous suggested that there might be hypertrophy of the fibrous stroma, such as is caused by injecting estrogens into male animals. Further evidence that the patient was secreting an excess of a hormone which behaved like an estrogen was afforded by the fact that an extract of his urine caused cornification of the vaginal epithelium in a castrate rat in a titer slightly greater than ordinarily found in a preadolescent or even in an adult male.

Although in one case the hormone produced by the adrenal tumor behaved like an androgen, and in the other like an estrogen, no information was obtained concerning the chemical structure of the steroids in the tumor tissue. The actual androgenic content of hyperplastic or neoplastic adrenal glands is very low despite the fact that they may give rise to excessive amounts of androgenic steroids in the urine (Crooke and Callow (4), Slot (5)). In our cases no attempt was made to isolate and identify the steroids in the small amounts of tumor tissue obtained. However, biologic assays for androgen and estrogen were made by Dr. Harry Hays, of the Ciba Pharmaceutical Products, Inc. on acetone and acetone-benzene-ether extracts of the tumors with negative results.

The study of the steroids of the urine has yielded more information than the analysis of tissue extracts. Not only are there increased quantities of these substances in cases of adrenal tumor or hyperplasia, but in some instances large amounts of abnormal steroids not found in normal urine are excreted (Wintersteiner (6)). It has been suggested by Talbot (7) that the finding of a large proportion of the beta fraction of the 17-ketosteroids is diagnostic of adrenal tumor. However, an increased beta fraction is not invariably found in cases of adrenal tumor and there may at times be some increase with hyperplasia (Dobriner (8)). Frank (9) found very high excretions of estrogens in some cases of virilizing tumor and considered that this was characteristic of adrenal carcinoma rather than adenoma.

In our patient with virilizing tumor, urine collected prior to operation contained from 19 to 22 mg. of 17-ketosteroids per day; and after metastases occurred, as much as 124 mg. (Specimens were sent to Dr. Konrad Dobriner of Memorial Hospital Research Laboratories, New York City, for isolation of the steroids but as yet no report has been obtained.) The boy with gynecomastia excreted only 4 mg. of 17-ketosteroids and insufficient urine was collected before operation to permit proper isolation of individual steroids. In a later section in which we review cases of feminizing adrenal tumor in adult males, it will be shown that there may be considerable variation in the steroid excretion in this condition. Using bioassay methods on the urine, Simpson and Joll (10), and Roholm and Teilum (11) demonstrated a marked increase of estrogenic and only slight increase in androgenic activity. We shall refer later to an unpublished case of feminizing tumor studied by W. W. Scott in which large amounts of beta 17-ketosteroids and only small amounts of estrogen were found in the urine. The biologic effects of abnormal steroids of the adrenal, found in large amounts only in pathologic conditions, is not fully understood.

The pathologic study of the two tumors added little to our knowledge. In each of them the cells were large, polyhedral and showed no particular structural arrangement. The protoplasm was deep-staining and granular, and did not show vacuoles. In fact, Dr. Blackman was able to detect no essential differences in the two cases and considered that each neoplasm resembled the prenatal zone of the adrenal, and was probably derived from it. The Broster-Vines (12) stain for fuchsinophilic granules did not aid in differentiation, as these granules were abundant in both cases. This differed from the experience of Simpson and Joll (10) who studied a malignant adrenal tumor causing gynecomastia in a male and compared it with a virilizing adrenal carcinoma in a woman. They reported that a different type of cell was found in the two tumors; and the virilizing tumor showed a "diffusely fuchsinophilic tinge." However, Sudds (13) concluded that the sex hormones play no part in determining the presence of fuchsinophilic granules in the adrenal. Blackman (1) came to the conclusion that all cells which stain intensely with eosin such as liver cells and the reticular zone cells of the adrenal, show granulation with the ponceau-fuchsin stain, whereas cells filled with lipoids are not stained.

CLASSIFICATION OF ADRENAL DISORDERS

The hormones of the adrenal cortex, their biologic effects and relations to pathologic conditions have been extensively discussed (6, 12, 14, 15, 16, 17, 18, 19, 20, 21). They fall into five groups of steroids differing in chemical structure and biologic activity: 1) corticosterones having no

oxygen attached to C_{11} which are concerned principally with the control of the electrolyte balance; 2) corticosterones with an atom of oxygen on C_{11} which influence the carbohydrate metabolism; 3) androgens; 4) estrogens and 5) progestins. Adrenal tumors may exhibit no hormonal manifestations or may cause symptoms attributable to the excessive production of one or of a number of different hormones (Cahill (18)). Excess of the glycogenetic cortins which tend to cause the utilization of amino acids for

TABLE 1. CLASSIFICATION OF DISEASES OF ADRENAL CORTEX ACCORDING TO HORMONAL MANIFESTATIONS

	Pathological Etiology	Hormonal Dysfunction Cortin	Androgen	Estrin
Addison's Disease	Adrenal TBC Adrenal Atrophy Pituitary Deficiency	−	−	
Cushing's Syndrome	Hypothalamic? Pit. Basoph. Tumor Adrenal Hyperplasia Adrenal Tumor	+++	+	− or ±
Adrenogenital Syndrome Female—Pseudohermaphroditism Male—Macrogenitosomia Precox	Embryonic Hyperplasia	N or − − (Frequent)	++ ++	
Virilization	Postnatal Tumor Postnatal Hyperplasia	N	+++ ++	
Feminization—Gynecomastia	Feminizing Tumor			++

glycogen formation and to inhibit protein anabolism, may account for the diabetic sugar curve, obesity, evidences of protein deprivation and other signs of metabolic disturbance encountered in Cushing's syndrome (Albright (19)). Occasional disturbances of the electrolyte balance and perhaps hypertension may result from excess production of adrenal cortins (McQuarrie (20, 21), Anderson and Haymaker (22)). Excessive elaboration of androgen leads to increased growth and muscular development, hirsutism, acne and virilization.

In view of the multiplicity of the hormones which may be elaborated, it is not surprising that adrenal disorders may cause a great variety of symptoms, or that there may be gradations between the adrenogenital syndrome and Cushing's syndrome. It would avoid confusion to define the *adrenogenital syndrome* as a disorder in which there are evidences of excessive production of adrenal androgen with either (a) no evidence of disturbed

TABLE 2. ADRENAL TUMORS IN CHILDREN 12 YEARS OR YOUNGER, CLASSIFIED ACCORDING TO HORMONAL MANIFESTATIONS

	Total Cases	Adreno-genital Syn-drome	Cushing's Syndrome				Femini-zation with Gyneco-mastia	Proven Malig-nancy	Operative Results		
			Obesity and Hirsut-ism	Obesity and Hirsut-ism with Early Female Develop-ment	Obesity only	Total			Explora-tion Only	Opera-tive Removal	Cured
Female	53	31*	14	6	2	22		21	4	28	11
Male	17	12	2		2	4	1	9	2	7	3
Total	70	43	16	6†	4‡	26	1	30	6	35	14

* Three cases were adrenal tumors of the ovary—Riche (36), Gaudier (35), Downes and Knox (34).

† Precocious breast development and/or menstruation in cases of Tilesius (37), Bullock and Sequeira (38), Walters, Wilder and Kepler (39), Friedgood and Gargill (30) and in two cases of ovarian adrenal tumor, Riche (36) and Gaudier (35).

‡ Females showing obesity without hirsutism were Gross's patients 4 and 5 (29)—both cases of short duration. Males were Lightwood's patient aged $4\frac{1}{2}$ months (32); and Taylor and Wiseman's (40) aged 11 months, who had a large penis.

cortin production or (b) deficiency of cortins:[1] and *Cushing's syndrome* as a disorder in which signs of excessive cortin production predominate, with usually some evidence of increased androgen and occasionally increased estrin. On the basis of these definitions the classification of adrenal disorders is shown in Table 1.

ADRENAL TUMORS IN CHILDHOOD

Cases of adrenal tumor in children have been collected from the literature by Reilly, Lisser and Hinman (26)—37 cases; Marks, Thomas and Warkany (27)—24 cases; Wilkins, Fleischmann and Howard (23)—11 cases in the male; and Goldstein, Rubin and Askin (28)—54 cases. Additional cases not included in these series have been reported by Gross (29), McQuarrie (20), Friedgood and Gargill (30), Pratt and Schaefer (31), Lightwood (32) and Wharton (33). Actually, excluding all duplications and adding our 2 cases, there are 70 children (53 girls and 17 boys) who developed adrenal tumor before the age of 12 years. In three of these cases, the neoplasm occurred in aberrant adrenal tissue in or near the ovaries (34, 35, 36). We have reviewed the published abstracts of these histories to determine which patients showed marked obesity, plethora, hypertension or other evidences of excessive cortin secretion, and which showed virilization with accelerated growth and muscular development suggesting only hypersecretion of androgen. The findings are shown in Table 2.

Of the 53 girls, 31 fulfilled our definition of simple adrenogenital syndrome, and 22 had symptoms suggestive of Cushing's syndrome. Of the 22 girls who were obese or had other manifestations of hypercorticism, all but 2 (29) who ran an unusually rapid course showed hirsutism or other evidence of excessive androgen, and 6 gave evidence of precocious secretion of estrin.[2]

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[1] The fact that embryonic hyperplasia of the adrenal causing macrogenitosomia praecox or female pseudohermaphroditism may lead to cortico-adrenal insufficiency has been discussed by Wilkins, Fleischmann and Howard (23), Bratrud and Thompson (24) and Jaudon (25).

[2] A 4-year old patient of Tilesius (37) was excessively fat and had large mammae and well developed pubic hair.

A patient of Bulloch and Sequeira (38) was obese and hirsute, had large breasts and menstruated at 10 years.

Walters, Wilder and Kepler's (39) patient developed breasts at 4 years. Her menses started at 8 years and recurred every 2 months. In addition, she showed marked hirsutism, acne, deep voice, advanced bone age, obesity and hypertension.

Friedgood and Gargill's patient (30) menstruated at 8 years, had evidences of marked virilization and also had plethora, striae and hypertension.

In addition, Riche (36) and Gaudier (35) each reported an adrenal rest tumor of the ovary causing menorrhagia and metrorrhagia at about 4 years.

It is of considerable interest that although evidences of virilization were manifested in 12 cases during the first year of life, and in a number of these within the first few months, abnormalities in the differentiation of the genital tract with persistence of a urogenital sinus did not occur in any case.[3] This is in marked contrast to congenital adrenal hyperplasia which in females practically always causes pseudohermaphroditism with a persistent urogenital sinus.

Of 17 males with adrenal tumor (20, 29, 32, 40, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55), 12 showed precocious sexual development, accelerated growth and osseous development and were muscular but not obese. Only 2 showed obesity of the Cushing's type (32, 40). Our patient (Donald G.) is the only case of feminization with gynecomastia in this age group. It should be noted that when precocious development of the male genitalia and secondary sex characteristics result from androgen derived from adrenal neoplasm or hyperplasia, the testes usually do not undergo adolescent development. In 6 cases the testes were described as small or normal, and in 7 cases they were not mentioned. Taylor and Wiseman (40) stated that in their 11-month old patient the testes were "larger than normal." In a 2½-year old patient of Fordyce and Evans (46), and a 3-year old boy of Macera (51) they were "large" but showed no sperm. In a 5-year old boy reported by Player and Lisser (52) the testes were said to be of adult size and erections and nocturnal emissions were reported. It is questionable whether spermatozoa were present. This is in marked contrast to the cases of males having sexual precocity due to brain lesions or to idiopathic premature activation of the pituitary, who usually develop mature adult testes with spermatogenesis.

The tendency of adrenal tumors to malignancy is shown by the fact that metastases occurred in 30 of the 70 cases. There is a high degree of operative mortality. Only 14 patients (11 girls and 3 boys) of the 35 in whom removal of the tumor was attempted were cured. The first successful operation was

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[3] Soffer (14) quotes incorrectly the cases of Scabell (41) and Krabbe (42) as instances of female pseudohermaphroditism associated with adrenal tumor. Actually, Scabell's first case was one of adrenal tumor causing virilization without genital maldevelopment and his second case was a female pseudohermaphrodite with congenital adrenal hyperplasia. Krabbe did not report any cases. He emphasized that adrenal lesions do not cause true sexual precocity but heterosexual development which he termed "pseudohermaphroditism." DaRocha (43) incorrectly applied the term "pseudohermaphroditism" to a case of virilization due to adrenal tumor in a 2-year old child. The case has been quoted as having the vagina "obliterated." Actually, there was a normal vagina obscured by a large clitoris. Bratrud and Thompson's (24) *case 7* was a female pseudohermaphrodite of 32 years who had bilateral congenital adrenal hyperplasia and also a carcinoma of the right adrenal which might have developed subsequently.

performed by Collett in 1924. A number of the patients died from hemorrhage and it is probable that postoperative shock and death were due in some cases to the fact that the contralateral adrenal may have undergone atrophy due to the excessive hormonal activity of the tumor so that adrenocortical insufficiency followed the removal of the neoplastic gland. It is probable that with improved technic and supportive adrenal therapy a higher percentage of cures will be obtained in the future.

FEMINIZING ADRENAL TUMORS CAUSING GYNECOMASTIA IN ADULT MALES

Ten previously reported cases of gynecomastia (10, 11, 56, 57, 58, 59, 60, 61) due to adrenal carcinoma in adults are listed in Table 3. We are grateful to Dr. W. W. Scott, Professor of Urology, Johns Hopkins Hospital, for his permission to add an eleventh case, hitherto unpublished, which he studied in 1945 in Chicago. All of these patients were between 26 and 44 years of age with the exception of the 15-year old patient of Holl (57). Our patient (D. G.) is the only case in which a feminizing tumor of the adrenal has been found before the onset of puberty.

The high degree of malignancy is shown by the fact that 9 of the 12 patients died from metastases or postoperative recurrences. Holl's *patient 2* was reported as cured after operation. McFadzean's (59) patient was well six weeks postoperatively. Our patient shows no evidence of recurrence or metastases four years after operation.

The breasts and nipples were well developed in all cases. Increased pigmentation of the nipples was noted in the two cases of Holl (57). In the case of zum Busch (61) a little milky fluid could be expressed, and in Lisser's case there was a watery secretion. All the patients apparently had some loss of libido and in 6 cases the testes were described as small or atrophic. In only two cases was there any tendency to obesity. Holl's 44-year old patient showed increased adiposity with the onset of symptoms, and lost weight following the successful removal of the tumor. The patient of Simpson and Joll (10) gained nearly 30 lbs. of weight during the first year after gynecomastia appeared, and then lost about 60 lbs. prior to operation. Following operation he regained his original weight but again lost when the tumor recurred. Hypertension or other evidences of Cushing's syndrome are not recorded in these cases. In Bittorf's (56) case there was increased pigmentation of the eyelids and face and in Holl's first case there was a pigmented linea fusca on the abdomen.

In only four cases have studies of the urinary hormones been made. In the cases of Simpson and Joll (10) and of Roholm and Teilum (11) bioassays showed only a slight increase of androgen but a large increase

TABLE 3. FEMINIZING ADRENAL TUMORS IN MALES

Author	Year	Age Yrs.	Breasts: Enlargement	Breasts: Secretion	Androgenic activity: Reduced Libido	Androgenic activity: Atrophy of Testes	Androgenic activity: Decreased Sexual Hair	Skin Pigment	Obesity	Androgen Bioassay	Urinary Assays: Estrogen Bioassay	Urinary Assays: 17-Ketosteroids	Outcome
1. Bittorf (56)	1919	26	+	0	+	+	0	+	0				Died—metastases
2. zumBusch (61)	1926	27	+	Milky					0				Died—metastases
3. Holl (57)—*case 1*	1930	15	+	0		0	+	+	0				Exploration—inoperable—died
4. Holl—*case 2*	1930	44	+	0	+	+	+	0	+				Operation—well
5. Lisser (58)	1936	33	+	Watery			0		0				Died—metastases
6. Simpson & Joll (10)	1938	34	+	0	+	+	+		+	50–100 e.e.u† per liter	3000 m.u./1		Operation—recurred—died
7. Pico Estrada (60) *Case 1*	1940	30	+	0		+	0	+	0				Died—metastases
8. Pico Estrada—*case 2*	1940	41	+	0			?	0	0				Died—metastases
9. Roholm and Teilum (11)	1942	44	+	0	+	+	+	0	0	5–80 e.e.u./24 hrs.	5000 m.u./24 hrs.		Died—metastases
10. McFadzean (59)	1946	29	+	0	+	0	0	0	0				Operation—well six weeks later
11. W. W. Scott		43	+	0	+	+	0	0	0		5.3 m.u./24 hrs. and estradiol	196 mg./24 hrs.	Operation—metastases—died
12. Wilkins		5	+	0		0		0	0		5 R.U./24 hours‡	4 mg./24 hrs.	Operation—well after four years

† e.e.u. =capon comb units. m.u. =mouse units.
‡ R.U. =rat units

of estrogen (5000 mouse units per day compared to less than 20 mouse units in normal individuals). W. W. Scott made extensive chemical studies of the steroids excreted by his patient. The total 17-ketosteroids amounted to 195.6 mg. per day. Of 189.8 mg. contained in the ketonic fraction there were only 5.8 mg. of alpha-ketosteroid while 70 mg. of crystalline dehydroisoandrosterone, a beta compound, were isolated. Estrogenic activity was equivalent to only 5.3 micrograms of alpha-estradiol benzoate per 24 hours. Following operative removal of the tumor the 17-ketosteroids decreased to 9.6 mg. per 24 hours with a reversal of the alpha-beta ratio. However, estrogenic activity was not decreased, being equivalent to 9.5 micrograms of alpha-estradiol benzoate per 24 hours. Analysis of the tumor showed no 17-ketosteroids although estrogens were found (data concerning the exact amount are not available). In our patient the excretion of 4 mg. of 17-ketosteroids per 24 hours was slightly greater than usually found at the the age of 5 years, but less than that of a normal adult. The estrogenic activity of 5 rat units per day represented only a questionable slight increase for a boy his age.

SUMMARY

A 5-year-old boy with marked gynecomastia was found to have an encapsulated adenoma of the adrenal cortex. After removal there was a gradual decrease in the size of the breasts and no evidence of recurrence after four years.

A 5½-year-old girl who had shown rapid virilization over a period of six months was found to have an encapsulated tumor of the adrenal cortex. After operation metastases recurred in the abdomen and the patient died two years later.

The feminizing tumor and the masculinizing tumor could not be differentiated histologically. In the case of gynecomastia the excretion of 17-ketosteroids was 4 mg. per day and small amounts of estrogen were demonstrated. The patient with virilization excreted 22 mg. of 17-ketosteroids before operation and 124 mg. after the tumor recurred. Less than 1 per cent consisted of the beta fraction.

Seventy cases of cortical adrenal tumor occurring in children under 12 years of age have been reviewed and classified according to whether they presented symptoms attributable only to excessive androgen or whether they also showed obesity and other metabolic disturbances suggestive of excessive production of cortin.

Ten cases of adrenal tumor causing gynecomastia in adult males are reviewed and an unpublished case with extensive hormonal studies by Dr. W. W. Scott is added. Our patient is the only instance of a feminizing adrenal tumor in a child.

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THE EFFECT OF STARVATION ON URINARY 17-KETOSTEROID EXCRETION*

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CHRONIC illness associated with malnutrition, debility and evidence of infection often leads to depression of urinary 17-ketosteroid values (1). Forbes et al. (2) report a rise in 17-ketosteroids following operations, fever or the sudden onset of an acute illness, followed by a decline after 12 to 48 hours which often reached subnormal levels. We have confirmed this in an unpublished study in a subject with induced fever in whom food intake was maintained unchanged. The numerous evidences, however, from studies on rats (3, 4) and dogs (5) that androgen production by the testes is reduced during inanition suggests that undernutrition alone may often contribute to the depression of 17-ketosteroid excretion in human disease. Accordingly the effects of starvation in three normal men and one obese woman were examined, together with those of relatively mild protein and caloric restriction in two normal men.

METHODS

The procedure differed somewhat among the subjects. The woman (H.F., age 30) and two of the normal men (R. L., age 27 and R. W., age 25) were on a constant, adequate, self-selected diet prior to starvation. One normal man (D. P., age 26) ate as he chose from day to day. Starvation in all instances lasted four days. R. W. was given 7 Gm. of salt daily in addition to water during this period. D. P. likewise received salt supplements; the others water only. The fast was terminated in all but R. W. by prompt resumption of the control diet. In R. W. four days of chiefly carbohydrate feedings (carb. 200 Gm., prot. 3 Gm., fat 0.6 Gm. daily) preceded resumption of the original diet. The men carried on their usual sedentary occupations throughout; the woman, although hospitalized, was up and about most of the time.

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In the protein depletion studies R. L. was shifted from an adequate control diet (carb. 282, prot. 108 (N 17.6), fat 158, cal. 2982) to a low protein diet with some caloric restriction (carb. 308, prot. 6.6 (N 1.1), fat 55, cal. 1755) for eight days and then returned to the original diet. A. K. (age 43) shifted from the control (carb. 285, prot. 86 (N 13.5), fat 148, cal. 2861) through two increasing grades of protein and caloric restriction before resuming the original diet. These were respectively carb. 343, prot. 29 (N 4.6), fat 85, cal. 2253 for six days and carb. 295, prot. 10 (N 1.7), fat 41, cal. 1589 for four days. The usual sedentary occupations were maintained.

In all subjects the 24-hour urine collections were checked for completeness by creatinine determinations (6). Urinary nitrogen (7) was determined daily except in D. P. Analyses for stool nitrogen were not made and the estimated nitrogen losses given are from urine only.

Total neutral 17-ketosteroids were determined daily. To confirm the course of the curves of excretion, 17-ketosteroids were measured in the ketonic fractions in numerous instances. Five hundred cc. aliquots of urine were hydrolyzed by boiling with 50 cc. of concentrated hydrochloric acid for 15 minutes, and then extracted with carbon tetrachloride for 2 hours in the extractor described by Consolazio and Talbott (8). The Zimmermann reaction was carried out by the method of Holtorff and Koch (9) in which aqueous potassium hydroxide is used. Androsterone served as a standard, except with H. F., when it was unavailable. Here dehydro-iso-androsterone acetate was used and the values were expressed in terms of dehydro-iso-androsterone. The color was allowed to develop for 45 minutes. Color corrections for non-ketonic chromogens were not made. Engstrom and Mason(10) have shown them to be inaccurate when applied in the Holtorff-Koch procedure.

For ketonic fractions, which are useful with the Holtorff-Koch procedure (10), separation from the crude material was accomplished by reaction with Girard's reagent T (11).

Since practice still differs considerably in detail among several laboratories measuring 17-ketosteroids, and since precise specificity is hardly claimed by any, the figures given have chiefly relative significance for the experiments at hand and will deviate somewhat in absolute amounts from those obtained by other methods elsewhere. The necessary change from androsterone to dehydro-iso-androsterone acetate as a standard in our own work presumably made some difference in the absolute values obtained (12).[1]

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[1] Androsterone and dehydro-iso-androsterone acetate were generously supplied by Dr. Irwin Schwenk of the Schering Corporation.

The androgenic activity of certain specimens was determined by measuring the growth of the capon's comb after local application of the neutral extract taken up in ether according to the method of Johnston and Koch (13). Ten birds were used in each assay. Androsterone served as the standard and the results are expressed in International Units (0.1 mg. androsterone per unit). This method, which is unpublished, may be considered roughly comparable to the injection method of Gallagher and Koch (14). As used here the data may be considered approximate only.

RESULTS

Fasting induced a progressive decline in the excretion of 17-ketosteroids in all 4 experiments (Figs. 1–4). This was always well defined by the third day and reached approximately 50 per cent of the baseline values by the fourth day. Spot determinations of the ketonic fractions paralleled those

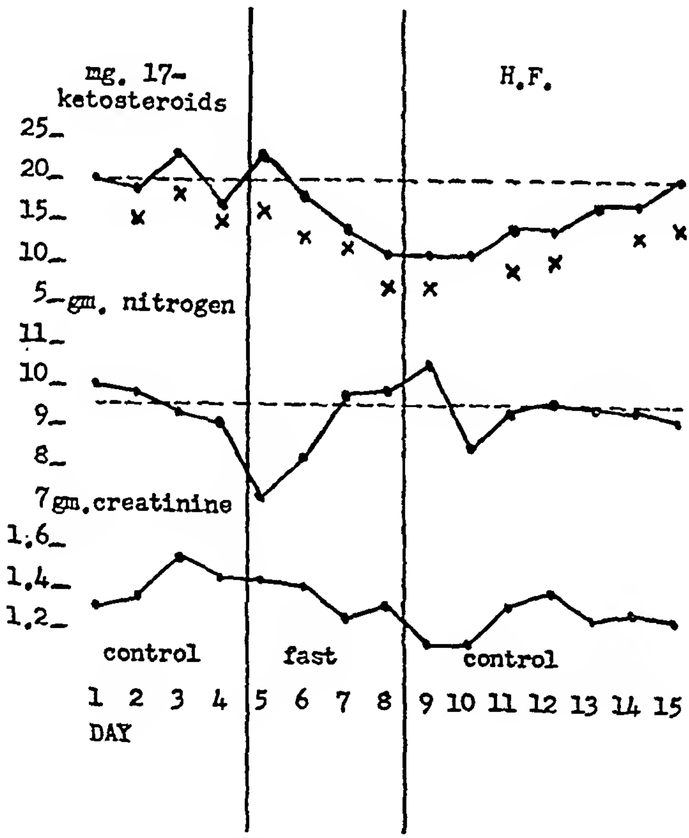

FIG. 1. The effect of starvation in a 30 year old woman on the urinary excretion of 17-ketosteroids, nitrogen and creatinine. Control diet: carb. 214, prot. 71 (N 11.35), fat 97, cal. 2013. x signifies ketonic fraction. The broken lines represent the average of control values.

of total neutral 17-ketosteroids. Supplements of salt during the fast made no difference in the results.

The course during recovery varied somewhat, depending upon experimental events. In H. F. and R. L. (Figs. 1, 2) 17-ketosteroids rose slowly and regularly to the control levels after 7 and 4 days respectively of normal

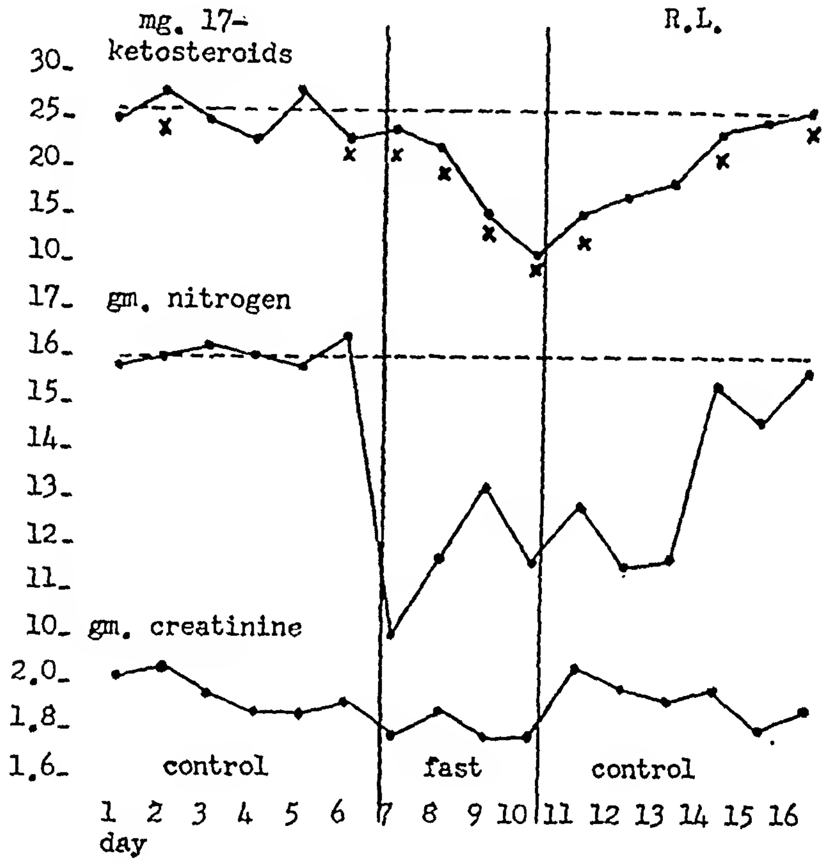

FIG 2. The effect of starvation in a 27 year old man on the urinary excretion of 17-ketosteroids, nitrogen and creatinine. Control diet: carb. 255, prot. 121 (N 19.3), fat 173, cal. 3061. x signifies ketonic fraction. The broken lines represent the average of control values.

feeding. In R. W. (Fig. 4) the consumption of 200 grams of carbohydrate daily to terminate the fast brought about only a slight recovery of 17-ketosteroid excretion although nitrogen excretion was sharply reduced. Full recovery to control 17-ketosteroid levels occurred only after 9 days of the normal diet. The sharp transient rise to 27 mg. in D. P. (Fig. 3) on the first day of refeeding may well have been due to the diodrast and insulin clearances, including painful catheterization, that were done as part of another study. Pincus' review (15) may be consulted for an account of the extensive work of his own group on the stimulating effects of psycho-

motor activity on 17-ketosteroid excretion. The discomfort and reaction in D. P. may well be analogous to certain elements in Pincus' experiments. Indeed, it is perhaps surprising that a phenomenon as labile as 17-ketosteroid excretion often seems to be, should prove so stable under the ordinary working conditions of our control studies.

The androgenic activity of selected samples of urine paralleled the 17-ketosteroid excretion in the three subjects studied. Since these data are not charted, they will be recorded briefly here. In R. L. the androgens fell from a prefasting level of 95 I. U. per day to 40 I. U. per day on the third and fourth days of the fast. Assays on the first and fourth days of refeeding

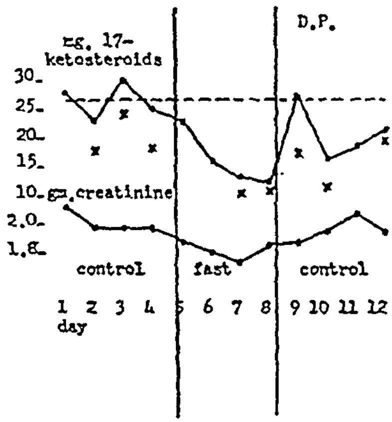

FIG. 3. The effect of starvation in a 26 year old man on the urinary excretion of 17-ketosteroids and creatinine. During the control periods the subject was on a free diet. x signifies ketonic fraction. The broken line represents the average of control values.

provided estimates in the neighborhood of 30 to 40 I. U. per day. On the sixth day of recovery a value of 75 I. U. per day was reached. In D. P. the initial value of 45 I. U. per day was reduced to 25 I. U. per day on the third and fourth days of the fast. In R. W. two prefasting estimates were 55 and 85 I. U. per day. Days 3 and 4 of the fast gave estimates of 30 and 15 I. U. per day respectively, and the fourth day of carbohydrate diet, only 30 I. U. per day. On the ninth day of refeeding an excretion of 50 I. U. per day was estimated. Since most of the androgenicity of normal urine is attributable to androsterone, these findings indicate a decline in the excretion of this steroid roughly paralleling that of total 17-ketosteroids.

The moderate nitrogen depletion produced in A. K. and R. L. by the low protein diets, somewhat restricted in caloric value (Figs. 5, 6), did not alter 17-ketosteroid excretion. Although the amount of nitrogen lost (A. K. 34 Gm. in 10 days; R. L. 43 Gm. in 8 days) approximated that lost by the fasting subjects (R. L. 46 Gm., R. W. 43 Gm., H. F. 35 Gm.,

each in 4 days) the physiological significance of the two types of nitrogen depletion may well differ sufficiently to mar comparison. In any event no evidence is adduced for any special sensitivity of the forces governing 17-ketosteroid production and excretion, to substantial reduction in protein intake.

Our attempt to measure testicular function indirectly during fasting by

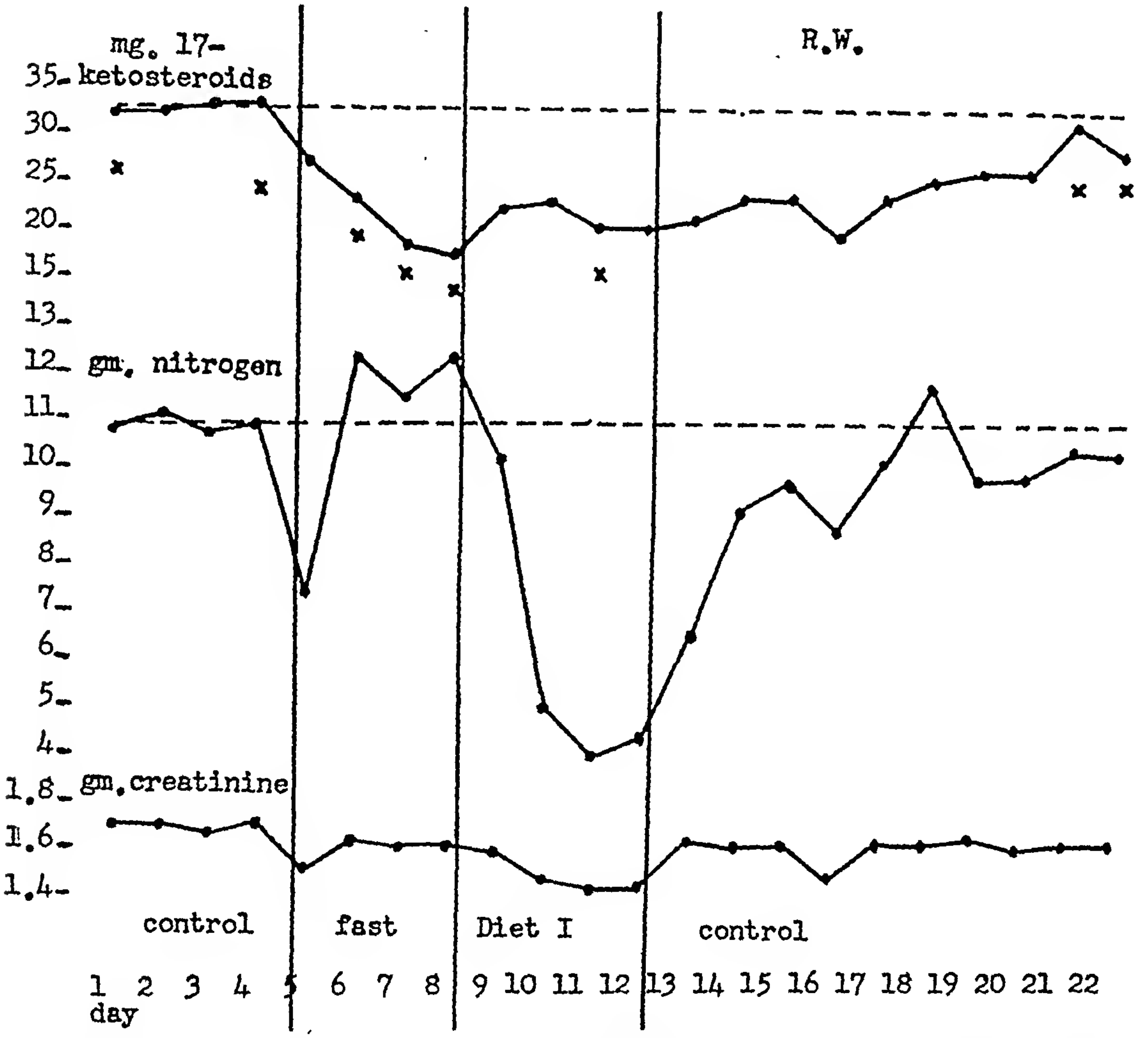

FIG. 4. The effect of starvation in a 25 year old man on the urinary excretion of 17-ketosteroids, nitrogen and creatinine. Control diet: carb. 308, prot. 84 (N 13.4), fat 125, cal. 2693. Diet I: carb. 200, prot. 3, fat 0.6, cal. 821. x signifies ketonic fraction. The broken lines represent the average of control values.

determining the urinary acid phosphatase excretion may be mentioned briefly, chiefly for the purpose of pointing up the necessity of developing corroborative measurements of testicular function when 17-ketosteroids are followed in the normal male. This enzyme is contributed in substantial part by the prostate and is excreted in the urine with some degree of con-

stancy in a given individual when discharge of seminal fluid is avoided. This phenomenon is indicated by a sharp peak in excretion which greatly exceeds the values usually obtained. In R. W. five control values ranged from 360 to 1050 (King Armstrong) units (avg. 592) per day. Values during the four days of fast ranged from 304 to 724 units (avg. 443); during the four days of carbohydrate feeding from 570 to 612 units (avg. 594);

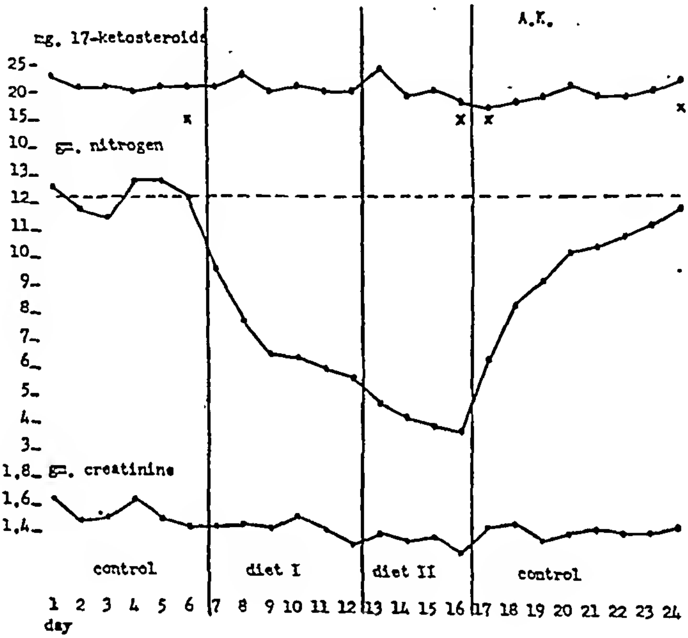

Fig. 5. The effect of low protein diets in a 43 year old man on the urinary excretion of 17-ketosteroids, nitrogen and creatinine. Control diet: carb. 285, prot. 86 (N 13.5), fat 148, cal. 2861. Diet I: carb. 343, prot. 29 (N 4.6), fat 85, cal. 2253. Diet II: carb. 295, prot. 10 (N 1.6), fat 41, cal. 1589. x signifies ketonic fraction. The broken line represents the average of control values.

and during the ten days after return to the original diet, from 320 to 712 (avg. 446), with no evidence of progressive change. In D. P. four baseline values ranged from 864 to 1159 units (avg. 1030); four during fasting, from 634 to 828 (avg. 748); and three during recovery, (excluding one of 3815, due presumably to seminal discharge) from 366 to 568 (avg. 491), with no progressive change. Little evidence of depressed testicular function during fasting can be extracted from such data. We are ignorant however of the time required for given degrees of testicular impairment to be reflected in prostatic involution, as represented by declining urinary phosphatase levels. It is thus by no means certain that our fasts were long enough to indicate diminished androgen production measured in this way.

COMMENTS

A consistent decline in the urinary excretion of total neutral 17-ketosteroids, confirmed by determinations of 17-ketosteroids in the ketonic fraction after Girard separation, was clearly defined by the third day of fasting in the three men and one woman studied. Estimations by biological assay indicated a parallel influence on the androgenic constituents. Such

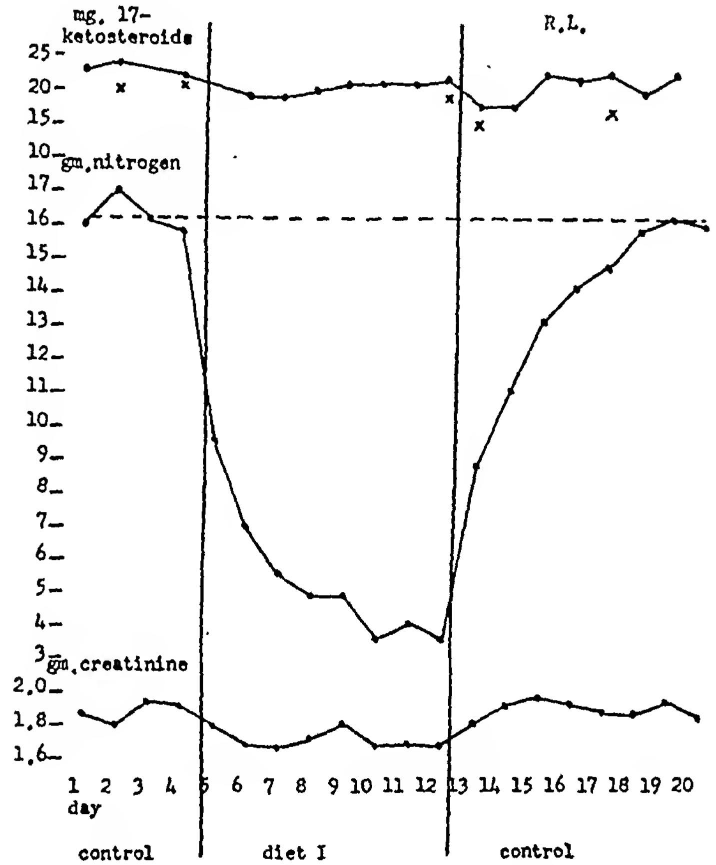

FIG. 6. The effect of a low protein diet in a 27 year old man on the urinary excretion of 17-ketosteroids, nitrogen and creatinine. Control diet: carb. 282, prot. 108 (N 17.6), fat 158, cal. 2982. Diet I: carb. 308, prot. 6.6 (N 1.1), fat 55, cal. 1755. x signifies ketonic fraction. The broken line represents the average of control values.

changes may be produced by any process which influences the rate of secretion of hormonal or other precursors concerned; the utilization of such hormones by receptive target cells; or the decomposition of such hormones and the conjugation and excretion of their resulting derivatives. Interpretations must accordingly be qualified by the uncertainties surrounding these events.

It is unlikely that the withdrawal of immediate dietary precursors is responsible. The low 17-ketosteroids in those patients with hypopituitarism and in women with Addison's disease who are eating normally or nearly so,

demonstrates that food does not inevitably supply 17-ketosteroids or their precursors. The possibility that food or a physiological substance such as bile, active in the handling of food, may contribute 17-ketosteroids only under the mediation of pituitary or adrenal secretions is without supporting evidence. Sharp reduction in protein intake did not induce the characteristic changes of starvation.

Inanition interferes with the inactivation of secreted estrogens by the liver (16), a process which may well be essential to the derivation and conjugation of steroids ultimately appearing in the urine. Should starvation similarly impair hepatic manipulation of the precursors of 17-ketosteroids, declining urinary excretion may reflect liver damage rather than altered secretion of hormones.

The testis, the adrenal cortex and possibly the ovary contribute hormonal precursors of 17-ketosteroids. In both rats (4) and dogs (5) starvation does eventually depress testicular androgen production. Since reactivity to gonadotropins is preserved, the testicular defect is presumably secondary to diminished pituitary stimulation. Such an effect would account very well for the reduced 17-ketosteroids in our men, although our attempts to corroborate such an explanation by following urinary acid phosphatase were hardly definitive.

The extent of ovarian contribution to urinary 17-ketosteroids in the normal woman is not entirely clear. It is now usually assumed that the relatively normal values observed some time after castration and the very low values in women with Addison's disease, in contrast to the less strikingly reduced values in men with Addison's disease, together demonstrate that the ovary, unlike the testis, makes no such contribution. Such considerations suggest that if the parallel decline in urinary 17-ketosteroid excretion during starvation in the two sexes is to have a common explanation it should be sought either in adrenal defect or in peculiarities of hepatic function rather than in the gonads. The experience of Scott and Vermeulen (17), however, deters us from pressing this point too firmly. In older men castration for prostatic cancer induced only a *temporary* decline in urinary 17-ketosteroid excretion. Until such carefully constructed experiments are available on women, we hesitate to use normal values some time after castration as fairly representing all phases of the response to loss of ovarian secretion. Our four day starvation, of course, would correspond in time to the early days after castration. To our minds for the moment, ovarian defect arising from starvation is not certainly excluded from responsibility for the declining urinary 17-ketosteroids of our woman.

It is possible in these experiments that depression of adrenal cortical secretion has occurred during starvation. In fasting rats hypertrophy of the adrenal cortex has been demonstrated (18), and interpreted

as meaning the participation of adrenal agents in the mobilization of glucose from tissue precursors (19). To the extent that such a process in man is reflected in urinary 17-ketosteroid excretion, one would expect an increase rather than a decrease during starvation. Many uncertainties, however, surround our understanding of the nature of the adrenal precursors of 17-ketosteroids in man. During starvation one would expect the enhanced secretion of a glucose-mobilizing, glycogen-depositing, nitrogen-expelling substance similar to corticosterone or its near relatives. Only recently Thorn (20) and Mason (21) and their associates have demonstrated rising urinary 17-ketosteroids after the administration of adrenotropins to man. In the experience of the Boston group the physiological effects were reminiscent of the properties of corticosterone or one of its relatives. The detailed studies of Venning and Browne (22), however, conducted largely after damaging events have not shown a strict agreement between the excretion of 17-ketosteroids and that of the clearly adrenocortical-like compounds of human urine. The suggestion (23, 24) that certain cortical precursors of normal 17-ketosteroids are anabolic and androgenic substances and hence unlike corticosterone or a near relative, should be kept in mind. To the extent that urinary 17-ketosteroid excretion reflects the activities of such a hypothetical agent, the physiological meaning in terms of nitrogen metabolism, for example, may be quite different from and indeed opposite to any reflection of the activity of a corticosterone-like compound.

Since possible precursors of 17-ketosteroids may exert either anabolic or catabolic influences on body protein, the relation between 17-ketosteroid excretion and the behavior of tissue protein in these experiments will be briefly summarized. No strict correlation between nitrogen excretion and 17-ketosteroid excretion appears from our data. Thus while in R. L. (Fig. 2) nitrogen excretion declined during fasting from the initial high values of a diet rich in protein somewhat in advance of the decline in 17-ketosteroid excretion, in R. W. (Fig. 4) urinary nitrogen rose above the control level as 17-ketosteroid excretion declined. Furthermore the termination of the fast by carbohydrate feedings in R. W. elevated 17-ketosteroids only slightly at a time when nitrogen excretion was sharply reduced and the rapid rise in nitrogen excretion on refeeding the original diet was followed only gradually by a rise in ketosteroids. In H. F. (Fig. 1) the later fasting values of urinary nitrogen corresponded with those of the control period, although 17-ketosteroid excretion was depressed. In A. K. and R. L. (Figs. 5, 6) the decline in nitrogen excretion on the low protein diets was unaccompanied by a distinct alteration in 17-ketosteroid excretion. Thus, although steroids with anabolic properties of a generalized character reduce urinary nitrogen excretion, no evidence from 17-ketosteroid excretion is

adduced here that they participate in any immediate and direct manner in those adjustments of the organism to variations in food consumption that are expressed by urinary nitrogen excretion. Nor do the variations in ketosteroid excretion fit the activities of a catabolic agent any better than they do those of a presumed anabolic agent. It is accordingly entirely likely that these variations are secondary and may be the result of pituitary inactivity induced by starvation, although they may represent processes that once expressed, are of some eventual metabolic value. It is possible, for instance, that any reduced anabolic force reflected in diminished 17-ketosteroid excretion during fasting would facilitate dissipation of tissue proteins by an active catabolic agent. Such a possibility is to be distinguished from immediate and direct responsibility for a process.

It is thus difficult to read a direct and simple interpretation of secretory phenomena from variations in 17-ketosteroid excretion in normal men and women subjected to starvation. In any event it is apparent that starvation is one factor in illness that will contribute to lowering urinary 17-ketosteroid values in either sex, and that restoration does not readily occur on small carbohydrate feedings only. On the other hand loss of nitrogen from the body induced by low protein diets with moderate caloric restriction has little effect on 17-ketosteroid excretion even when the amount lost approximates that due to starvation. It is possible that the rate of nitrogen discharge or the differing source and physiological significance of that discharged determines the difference in response of 17-ketosteroids. More extensive protein depletion might well, of course, provide a response not indicated in these brief experiments.

SUMMARY

1. In three normal men and in one obese woman starvation for four days produced a decrease of 50 per cent in the excretion of total neutral 17-ketosteroids and of those in the ketonic fraction after Girard separation. The effect was well defined by the third day. With restoration of a normal diet the values returned to their previous levels in about a week. In those studied (men), urinary androgen excretion was found to parallel that of 17-ketosteroids.

2. In two normal men no significant alteration in 17-ketosteroid excretion was produced by a diet low in protein and somewhat restricted in calories within the 8 to 10 day period of study.

3. More precise information concerning the source and metabolic pathways of the precursors of urinary 17-ketosteroids must be forthcoming before exact interpretation is possible.

4. Inanition is one factor that will cause a lowering of urinary 17-ketosteroid excretion in disease.

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A COMPARISON OF ORAL AND VAGINAL EPITHELIAL SMEARS

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THIS study was undertaken for the purpose of comparing the relative merits of oral and vaginal epithelial smears in an attempt to simplify or supplement the clinical method of appraising the endocrine changes during the ovarian cycle in women. Vaginal smears have been shown to be useful in studying the menstrual cycle, and it was felt that similar changes might be reflected in the oral mucosa since the latter possesses certain features in common with the vaginal mucosa, when examined histologically. For example, both areas may simultaneously present signs of leukoplakia, lichen planus, ulcers, and other diseases. On the other hand, there are differences. The oral mucosa is composed of more layers of cells than the resting vaginal mucosa; the outer basal cell layer and the intermediate and superficial cell layers of the vagina do not have exact counterparts in the oral epithelium; the cornified cells, when present, comprise the most superficial layer in the oral epithelium while in the vaginal epithelium the intermediate layer sheds the cornified cells. Despite these differences, if it could be established that the oral mucosa undergoes rhythmic changes as does the vaginal mucosa, the oral smear might prove to be another means of indicating certain hormonal conditions. In some cases, this method could present advantages over the vaginal smear as it is a simpler operation, involves fewer technical difficulties, could be applied to both men and women, and does not arouse the negative emotional response which often hampers or contraindicates the taking of vaginal smears.

Observations were made on a group of women complaining of orolingual pain. These patients were placed on estrogen therapy and were studied in connection with the orolingual problem, which is reported elsewhere (1). This group, plus others in estrogen deprivation states, also provided the experimental material for the present report, on the theory that the process of epithelial restoration could be more readily followed by means of oral and vaginal smears in a series receiving substitution therapy than in women with normal estrogen function.

We were guided by Papanicolaou's original work on vaginal smears (2) together with later amplifications by Papanicolaou and Shorr (3) (4) and others (5,6, 7, 8, 9). The conclusion to be drawn from these studies is that the proper use of daily vaginal smears enables the investigator to follow

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major deviations in ovarian hormone functions as well as important changes during the menstrual cycle.

In order to emphasize the status of hormonal action on the oral mucosa, a summary of certain experimentation is interjected before describing the present problem further.

SOME PREVIOUS STUDIES OF HORMONAL EFFECTS ON ORAL MUCOSAE

One of us (Ziskin) studied the effects of ovarian and other hormones on the oral mucous membranes.[1] Estrogens were injected into rhesus monkeys

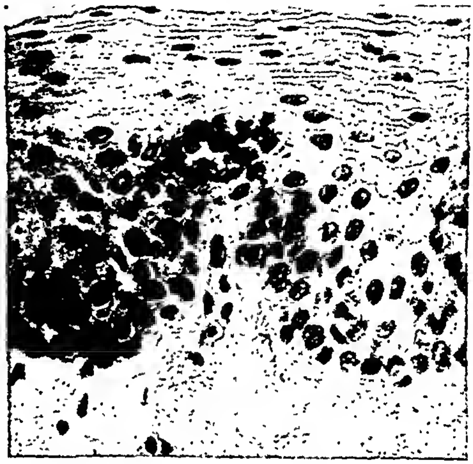


Fig. 1. Monkey 209. Female macacus rhesus. Normal mucous membrane. The keratin layer is absent, as in the human tissue. ×750.

Note: All tissue sections (Figs. 1 to 13, inclusive) are from female macacus rhesus monkeys weighing from 2 to 4 kilograms. The term "gingival," as used here, refers to the hard, pink gums; the term "mucous membrane," to the tissue immediately beyond the hard, pink gums and extending to the muco-buccal reflection; the term "cheek mucous membrane," to the lining of the cheek.

in the following categories: 1) castrated, 2) hypophysectomized, 3) normal immature, 4) sub-adult, 5) adult female, and 6) adult male. In all six groups the clinical result of estrogen injections was the production of gingival hypertrophy. This was seen by an amelioration of gingivitis, if present, and by a thickening and blanching of the gingivae.

The usual gross dermal changes, such as reddening and wrinkling of the sex skin, were observed. Microscopically, the gingival changes seen clinically proved to be hyperplasia (Figs. 16 and 17). Normal oral mucous membranes do not show surface keratin as do the gingivae (Figures 1, 14, 15); yet, after the estrogen injections, there was a definite layer of keratin on all mucous membranes (Fig. 6). The normally keratinized gingivae became hyperkeratinized as evidenced by a thickening and maturation of the sur-

[1] See References (10, 11, 12, 13, 14). This review is included as background because it presents certain factors influencing the epithelial structures of the oral mucosae. Since the major interest here centers around the absence, presence or modification of the keratin layer, the photomicrographs included were selected to illustrate these conditions.

face keratin in some (Fig. 17), and the formation of epithelial pearls in the prickle cell layer (Figs. 5, 16, 17). Mitosis was slightly increased.

The described changes were seen in all categories, proportional to dos-

FIG. 2. Monkey 214. 2½ months castrate. Mucous membrane. Surface keratin is absent. The prickle cells are smaller than normal, irregular in size, hyperchromatic, and pyknotic. The nuclear membranes are prominent. Compare with Fig. 1. ×400.

FIG. 3. Monkey 233. 8 months castrate. Mucous membrane. Fragmentation, pyknosis, and lysis of cells; and generally marked tissue alteration of a degenerative nature. The changes are more severe than in Fig. 2. ×540.

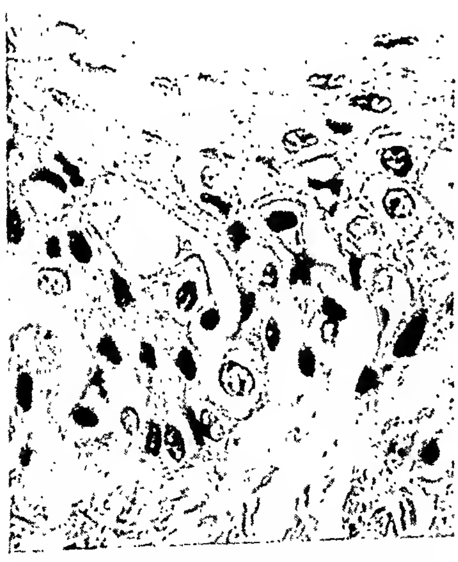
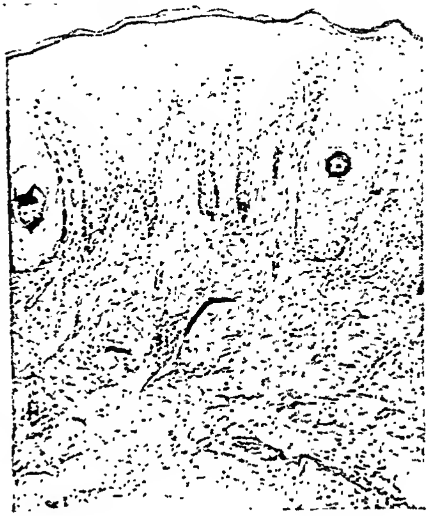


FIG. 4. Monkey 238. Hypophysectomized (complete) 47 days. Mucous membrane. Shows pyknosis, lysis of cells, fragmentation of tissue. Similar but more emphatic changes than seen in Fig. 3. ×1340.

age (within the limits of the capacity of the tissues), and were most prominent in those animals under treatment for the longest period of time.

To test further the estrogen effect, pyridine extracts of the anterior pituitary gland, an extract of the urine of castrated women, and an extract from blood serum of pregnant mares (equine gonadotropin), all known to have an ovarian follicle stimulating effect, were injected into

Fig. 5. Monkey 208. Castrate. Shows the gingivae after the injection of 135,000 R.U. estrogen in 79 days. Note the hyperplasia of both the epithelium and connective tissue and the presence of epithelial pearls. ×150.



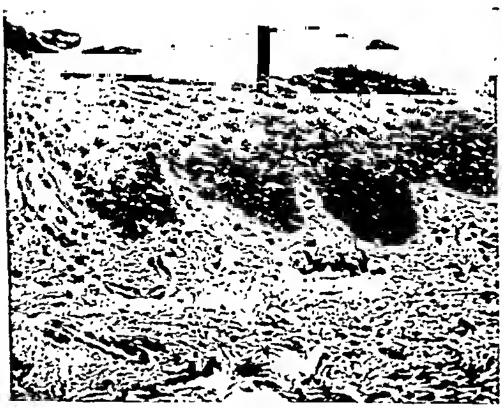


Fig. 6. Same animal as in Fig. 5. Cheek mucous membrane. Shows hyperplasia and presence of a keratin layer where none is seen ordinarily.

baby monkeys, into young mature monkeys and into hypophysectomized monkeys (completeness of hypophysectomy demonstrated by serial sections of the capsule). In all, the characteristic estrogenic changes described above were induced (Figs. 7 and 8).

The oral mucous membranes of castrated and hypophysectomized monkeys without estrogen replacement were also considered. In the former, the changes varied in degree, depending on the length of the period of castration. The whole tissue stained faintly and became fragile, being torn easily while sectioning. The normal keratin-bearing areas showed parakeratosis, thinning, or loss of surface keratin. The prickle cells lost their horizontal arrangement. The cells were columnar in shape and

disposed in vertical rows. The intercellular spaces were widened, giving them undue prominence. The nuclei were shrunken and pyknotic and a moth-eaten appearance was seen in many areas. The basal cells were small,

FIG. 7. Monkey 210. Shows the gingivae after treatment with gonadotropic hormone extracted from the urine of castrated women. (13,800 cc. urine equivalent injected in 37 days.) The changes are hyperplasia and hyperkeratinization. Note similarity to Figs. 5, 16, and 17. ×150.

FIG. 8. Monkey 487. Mucous membrane. Hypophysectomized (complete). Treated with 5,675 units of an extract containing the gonadotropic hormone of pregnant mare serum (equine gonadotropin) over a period of 32 days. Note the presence of an abnormal layer of keratin on the surface. ×1020.

irregular in size, with hyperchromatic nuclei. The whole tissue was narrower (Figs. 2 and 3). The changes following hypophysectomy were similar to the foregoing, but more severe (Fig. 4).

Several factors were found to modify the estrogen effect on the oral tissues of monkeys. Prominent among these was chorionic gonadotropin. Microscopically, the keratin layer of the epithelium appeared diminished in thickness or absent entirely. A zone of parakeratosis was sometimes seen in this area. Extensive hydropic change was seen in the prickle cell layer, with pyknotic nuclei toward the surface. Hyperplasia of the germinal layer was observed. The rete cones were elongated, pointed, and split frequently (Figs. 9 and 10). The chorionic gonadotropin may be partly re-

FIG. 9

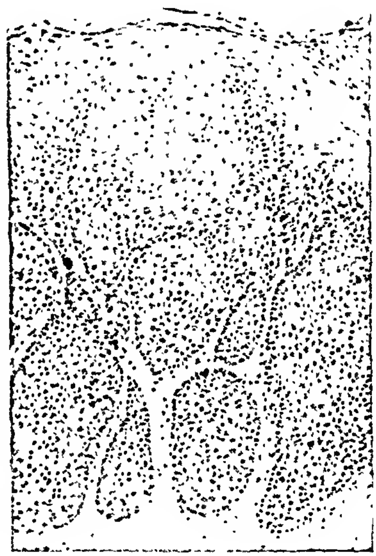


FIG. 10

FIG. 9. Monkey 123. The pre-experimental gingivae. ×300. See Fig. 10.

FIG. 10. Gingivae of same animal as in Fig. 9 after treatment with a crude extract of pregnancy urine (chorionic gonadotropin) for 68 days. The change consists of loss of surface keratin replaced by a parakeratosis. Marked hyperplasia is also seen. ×160.

sponsible for similar changes in the oral mucosa of pregnant women (15), (Figs. 21 and 22).

The effects of progesterone in both castrated and normal female monkeys were also tested. The epithelial changes consisted chiefly of a diminution or alteration in the keratin layer and an altered appearance of the prickle cells, mainly in the upper half of the layer. In both groups, prickle cell nuclei were pyknotic, shrunken, irregular in size and outline, and, in some instances, fragmented. The changes noted were more extreme in the castrated animals.

These experiments showed that estrogens in large quantities produced hyperkeratinization of the oral mucous membranes while other internal

secretions or their absence resulted in modifications of the keratin formation.

Changes in gingivae and other oral mucous membranes, similar to those seen in the monkeys, were found in the biopsies of a group of women receiving estrogen injections for gynecological disorders (Figs. 16 and 17).

Oral biopsies from clinical cases with other endocrine disturbances substantiated this evidence. For example, in a patient with Addison's disease a hyper-estrogen condition is suggested, the altered estrogen metabolism affecting the surface keratin (Fig. 18). Conversely, in a child of 7 years with

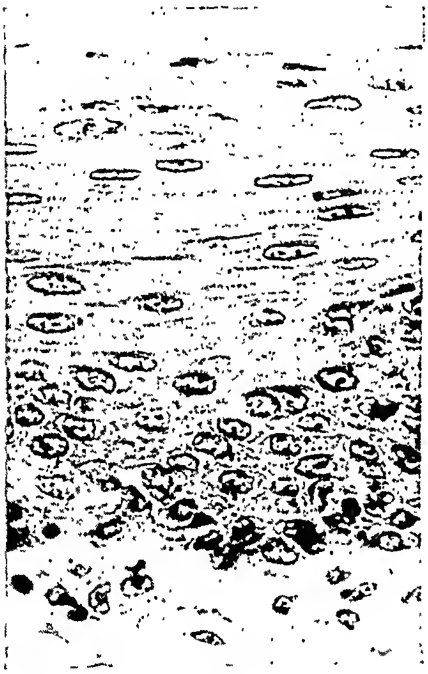


Fig. 11. Monkey 471. Cheek mucous membrane of normal animal injected with 92 mg. of progesterone in 30 days. Shows irregular outline and pyknosis of surface cells, and increased prominence of nuclear membranes. Outline of nuclei in germinal cells is also irregular. ×1000.

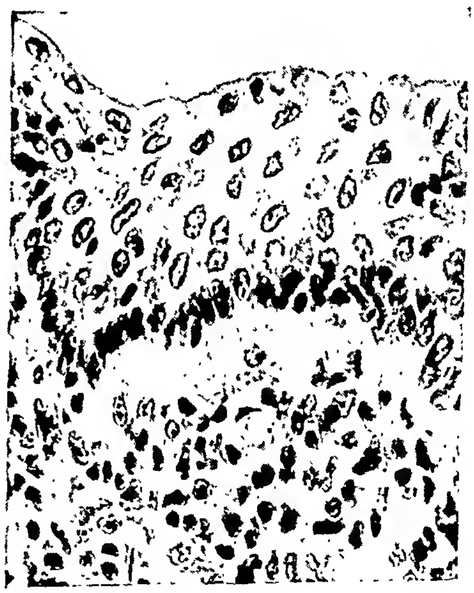


Fig. 12. Same animal as in Fig. 11. Vaginal mucous membrane. Shows irregular outline of nuclei and increased prominence of nuclear membranes. ×1000.

adrenal hyperplasia, pseudohermaphrodism, hirsutism and accelerated osseous development, the gingivae showed a lack of normal surface keratin (Fig. 19). This lack was also noted in a female, age 21, with glandular dystrophy (of unknown etiology), hirsutism and secondary amenorrhea of recent origin (Fig. 20).

The inferences drawn from this research suggested that stained scrapings

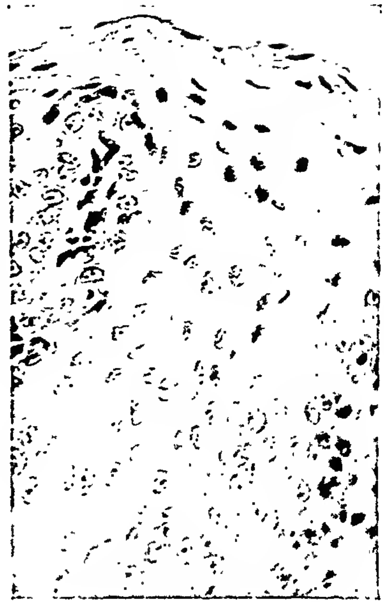


FIG. 13. Monkey 459. Castrate. Injected with 63 mg. of progesterone in 31 days. Gingival section shows irregular outlines of prickle cell nuclei. ×710.

FIG. 14. D. W. Female, age 26. Normal gingivae, 18th day of menstrual cycle. This photomicrograph is included to illustrate the histology of the normal human gingivae. The normal monkey's gingivae are similar except that the layer of granulosa cells is absent. ×117.

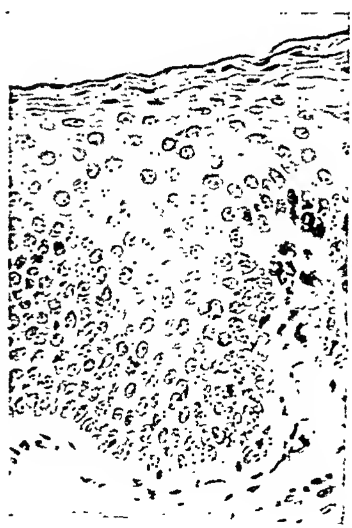


FIG. 15. C.S. Male, age 24. Normal mucous membrane. The keratin layer is absent, the surface being composed of flattened nuclei. ×560.

FIG. 16. B.H. Female, age 31. Gingivae, *after injections of 85,000 R.U. estrogen in 8* weeks for the treatment of secondary amenorrhea. The tissue shows hyperplasia, hyperkeratinization and epithelial pearls. ×160.

FIG. 17. B.H. Female, age 41. The gingivae after diethylstilbestrol therapy for 2 years for a gynecological disorder. Note the presence of a heavy keratin layer on the surface, hyperplasia, and epithelial pearls. ×175.

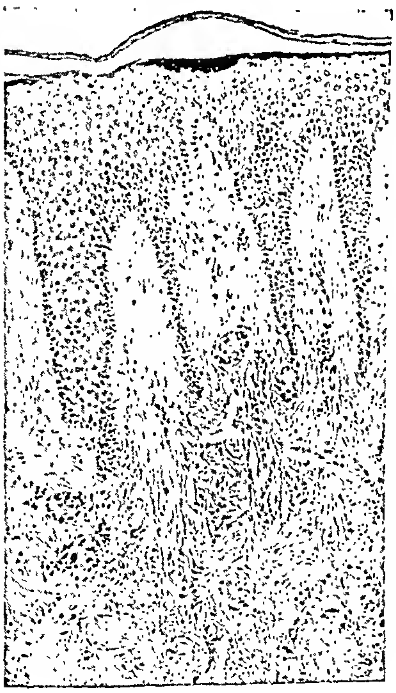


FIG. 18. J.R. Male, age 29. The gingivae from a case of Addison's disease. Hyperplasia and hyperkeratinization are in evidence. The implication is that because of adrenal cortical insufficiency, the modification of the estrogenic effect is absent, thus making more endogenous estrogen available to the gingivae. ×160.

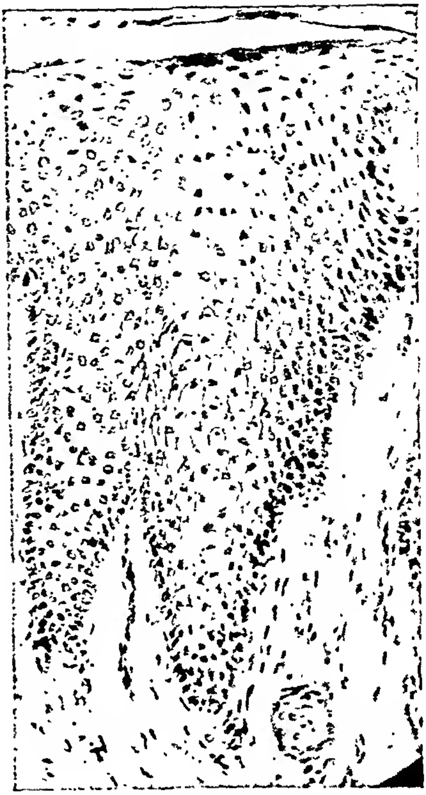


Fig. 19. C.A. Male, age 7. A case of adrenal hyperplasia and pseudohermaphroditism; bone age 25 years; infantile ovaries and uterus removed; no testicular tissue found; hirsutism and enlarged clitoris gave masculine appearance. The gingivae show absence of surface keratin. The round pyknotic nuclei seen on the surface together with the deeper-staining cytoplasm may constitute parakeratosis. Pyknotic nuclei and hydropic change appear in the prickle cell layer. The basal cells are small, irregular and widely spaced. The implication is that the excessive cortical secretion has a modifying effect on the endogenous estrogen so that the estrogenic effect on the gingivae is reduced or absent. ×336.

←

Fig. 20. A.T. Female, age 21. A case of hirsutism with endocrine gland dysfunction of unknown origin, and secondary amenorrhea of recent origin. The gingivae show an absence of surface keratin which may be attributed to a modification of the available endogenous estrogen. ×224.

from gingival surfaces might be useful in evaluating endogenous estrogen levels, and this hypothesis constitutes the basis for the present comparative study.

METHODS

The oral epithelial smears consisted of scrapings taken from the surface of the cheek mucosae and from the keratinized gingivae by lightly passing a thin flat metal blade (spatula) over the tissues.[2] The vaginal smears

[2] There are various levels of keratinization in the mouth: the hard palate would rate 4 plus; the hard pink gums, 3 plus; and the cheek mucosa (which normally does not have

were made by inserting into the vagina a glass tube to which was attached a rubber bulb, as described by Papanicolaou (2). The cells shed from the vaginal walls were thus drawn into the tube.

The cells from both areas were spread on glass slides and fixed in alcohol-ether (50–50). First, the oral smears were stained by Weinman's method

TABLE 1. STAGES OF CORNIFICATION AND CELL TYPES IN ORAL AND VAGINAL* EPITHELIAL SMEARS

Stage of Cornification	Percentage of Cornification	Predominant Types of Cells and General Appearance of Smear
Stage 0 (Atrophic smear)	None	Pale blue, non-cornified cells with large vesicular nuclei predominant. Many small basal cells with blue cytoplasm in vaginal smear.
Stage I (Resembles menstrual or early postmenstrual smear)	1–15%	Many non-cornified cells with some cornified forms with purplish cytoplasm and some smaller nuclei. Cells generally clumped. Many W.B.C. and cellular debris in vaginal smear.
Stage II (Resembles follicular or copulative smear)	15–40%	Many cornified red cells with small dark pyknotic nuclei. Remaining cells purplish, precornified forms.
Stage III (Maximum estrogen response. Resembles ovulatory smear)	40–95%	Most cells cornified. Cells distinct and separate. Vaginal smear "clean" with minimum number of W.B.C.
Stage IV (Resembles estrogen decline in the progestational phase)	80–40%	Both cornified and noncornified cells. Fragmented, anucleated, degenerative forms. In vaginal smear, cellular debris and W.B.C. return.

* Compare description of cells with that of DeAllende, I.L.C., Shorr, E., and Hartman, C. G., "A Comparative Study of the Vaginal Smear Cycle of the Rhesus Monkey and the Human," Carnegie Institution of Washington. Publication 557. Contributions to Embryology. XXXI, 1–26, December 22, 1943.

(16), using the the Ernst modification (17) of the Gram stain; and the vaginal smears were given the Shorr modification of Masson's trichrome stain (18). Later, the Gram stain was discarded and the Shorr method was used for both.

The oral and vaginal smears were taken simultaneously from normal

a definite keratin layer) occasionally 0 to 1 plus. In this series, scrapings from the cheek were used wherever possible because they took the Shorr stain as effectively as the vaginal cells and, therefore, facilitated comparison. Gingival smears were taken when indicated.

menstruating women. Readings were made by each of us, independently, and then compared and uniformly recorded. After ascertaining that the major changes in cornification of the vaginal smear were reflected in the oral smear, a series of cases in various endocrine states was studied in greater detail.

In cases where the menstrual cycle was absent, changes in cornification resulting from estrogen or other therapy were recorded in stages graded I,

TABLE 2. EFFECT OF ESTROGENIC TREATMENT ON EPITHELIAL SMEARS OF TEN POSTMENOPAUSAL WOMEN*

Case	Age Years	Menopause Years	Stilbestrol mg.	Benzestrol mg.	Estradiol Benzoate rat units	Stages of Cornification: Before Treatment		During Treatment		After Treatment	
						Vag.	Oral	Vag.	Oral	Vag.	Oral
No. 7, A.G.	54	5.5	21				I		II	I	I
No. 8, C.H.	56	15	50				0	III	II		
No. 9, A.A.	55	10	46				I	II	II		I
No. 10, R.S.	56	9	12				I	III	II		I
No. 11, S.Y.	52	3	9		40,000	II	III	III	II		
No. 12, B.H.	56	10	16	56		I	0	III	I		0
No. 13, E.C.	55	12	13	75			I	III	I		I
No. 14, F.S.	58	8		200	100,000		I	III	I		I
No. 15, B.P.	58	6		80	40,000	I	I		II		I
No. 16, J.E.	54	½		80	90,000	I	I	II	II	I	I

* Stilbestrol was given in doses of 1 mg. daily; benzestrol 2 mg. daily; and estradiol benzoate 10,000 rat units weekly. Duration of treatment may accordingly be estimated from the total doses listed.

II, III and IV, since the phases of the menstrual cycle did not exist. The cornification stages are explained in Table 1 and illustrated in Figure 23.

EXPERIMENTAL PROCEDURE

Oral and vaginal smears were compared in a group of 23 women. *Case 1* was that of a girl with secondary amenorrhea; *Case 2* was a normal pregnancy; *Cases 3 to 6*, inclusive, were in menstruating women with fairly normal cycles; *Cases 7 to 16*, inclusive, comprised ten middle-aged women, all menopausal, presenting the complaint of oral burning (tongue or gums) or dryness of the mouth and throat; and seven women of menopausal age (*Cases 17 to 23*, inclusive) without oral complaints, to serve as controls for the ten cases of glossodynia.

The vaginal and oral smears, treated with the Shorr stain, presented certain differences as well as some features in common. The dissimilarities were as follows:

1) No basal cells were seen in the oral smears.

2) The cytoplasm in the precornified oral cells showed purple granules and stained more deeply than the vaginal. The oral cells and nuclei were smaller than those in the vaginal smears and the nuclei were more pyknotic. (The oral precornified cells are the counterpart of those vaginal cells characteristic of the copulative phase of the menstrual cycle.)

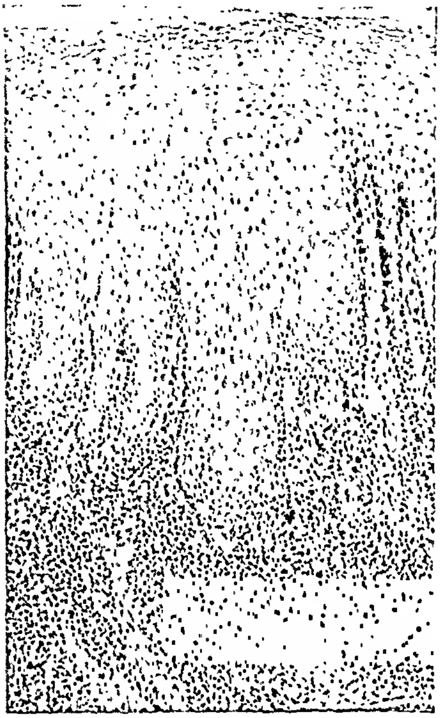


Fig. 21. B.E. Female, age 27. Eighth month of pregnancy (multipara). A section from the gingivae. Note loss of surface keratin, hyperplasia, downgrowth of rete cones, and inflammatory exudate in lamina propia. ×150.

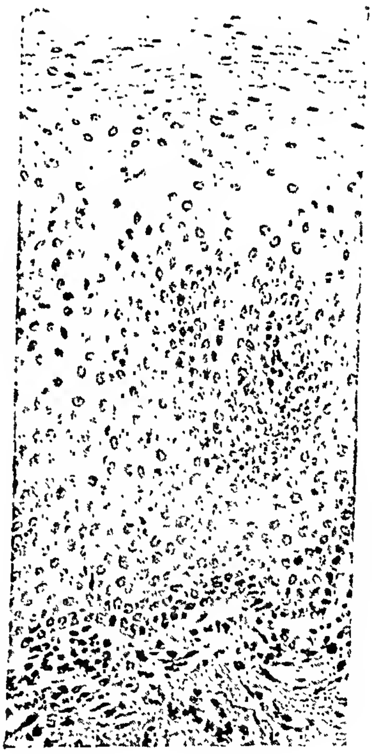


Fig. 22. Same case as in Fig. 21. A section from the mucous membrane. Changes are similar to those in Fig. 21. ×336.

3) The white blood cells seen mostly at ovulation in the vaginal smear were rarely found in the oral smear.

The following are the main points of similarity:

1) The non-cornified cells of the oral smears showed a pale grey-blue cytoplasm with large vesicular nuclei resembling the vaginal cells found most plentifully in the menstrual and postmenstrual smears.

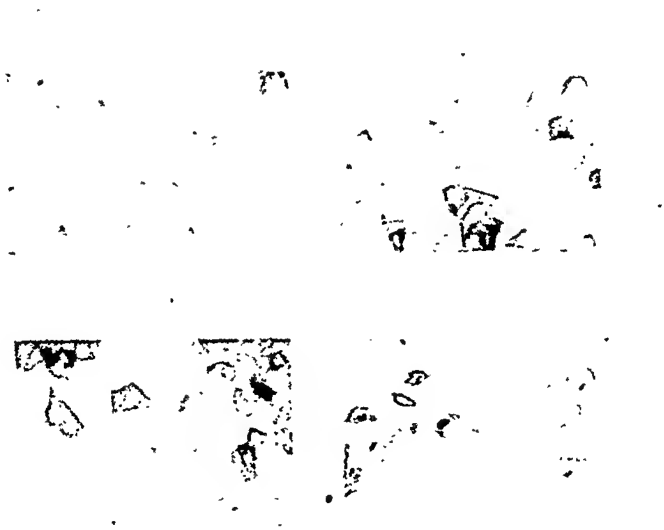


FIG. 23. Oral epithelial smears stained with the Shorr trichrome stain and illustrative of the four stages in cornification. Stage I: menstrual and postmenstrual phase. Stage II: copulative phase. Stage III: preovulatory and ovulatory phase. Stage IV: estrogen withdrawal phase; and degenerative forms.

2) The fully cornified oral cells were for the most part seen to be discrete and red (eosinophilic). Some had dark, pyknotic nuclei, others were anucleated, and still others showed a vacuole in the center where the nucleus had dropped out, or a clear halo around a degenerated nucleus. (These oral cells are the counterpart of the vaginal cells characteristic of the time of ovulation. They are found most commonly in scrapings from the gums.)

3) The postovulatory or progestational phase of the cycle was typified in both groups by degenerated, folded, and fragmented cells, usually clumped together (Fig. 23). (The histological sections from progesterone treated monkeys (Figs. 11, 12, 13) also show distorted and fragmented cell outlines and may explain the forms described here.)

PROTOCOLS

Case 1. G. A., a 19-year-old girl with secondary amenorrhea, was studied for seven months by oral and vaginal smears. Her amenorrhea was of three years duration, the menstrual function having been fairly normal from the thirteenth to the sixteenth year. Medical study, with special emphasis on the metabolic and endocrine aspects, failed to reveal any physical factors other than malnutrition resulting from anorexia. There were evidences of psychological factors as possible causes of the amenorrhea. Most of the vaginal smears showed low cornification of from 5 per cent to 15 per cent, and resembled smears taken during the postmenstruation period when there is little estrogen effect. There were two notable exceptions: cornification of 40 per cent was seen on April 11 and June 26. Cornification was likewise low in all of the cheek smears with four exceptions: April 11, May 3, June 17, and June 26, which were Stage II cornification. It is significant that on two of these dates, April 11 and June 26, the spectacular rise in cornification seen in the oral smears coincided with highly cornified vaginal smears. The gums throughout were swollen, soft, and spongy. They bled easily, and the surface epithelium was readily shed. This was accounted for by low cornification and, probably, depressed estrogen effect. Scrapings for the epithelial smears were taken with ease in contrast to the usual difficulty encountered in cases of hard, firm gums. Although the cause of the cornification peaks is obscure, it is pertinent that both mucosae were affected simultaneously in these two instances.

Case 2. R.F., a woman 29 years of age. An uncomplicated pregnancy was studied by means of vaginal and oral smears taken at weekly intervals from the first month until five weeks after delivery, except that during the crucial two weeks starting the day before delivery they were a daily procedure. The vaginal smears seen during the 10-day period following delivery were obscured by blood. All others were satisfactory.

In the first month of pregnancy, the vaginal smears showed a cornification of from 15 per cent to 20 per cent (Stages I and II), and were "dirty" with many white blood cells and debris. As pregnancy progressed, cornification fell, until by the sixth month cornified cells were rare; but they increased to about 1 per cent during the two weeks before delivery. The vaginal smears became cleaner, especially during the seventh and eighth months, but debris and white blood cells increased again in the last month. During the first eight months the cells became progressively smaller and more folded and wrinkled, with an increasing predominance of the "oyster" and "navicular" cells typical of pregnancy. Five days before delivery the cells appeared plumper; the day before delivery, at the onset of labor, they were vesicular. Little could be ascertained from the vaginal

smears for several days after delivery, except that cornification was low. On the ninth day post partum, cornification suddenly increased to about 10 per cent and remained at approximately that level for the balance of the experimental period. Basal cells were seen during the third post partum week.

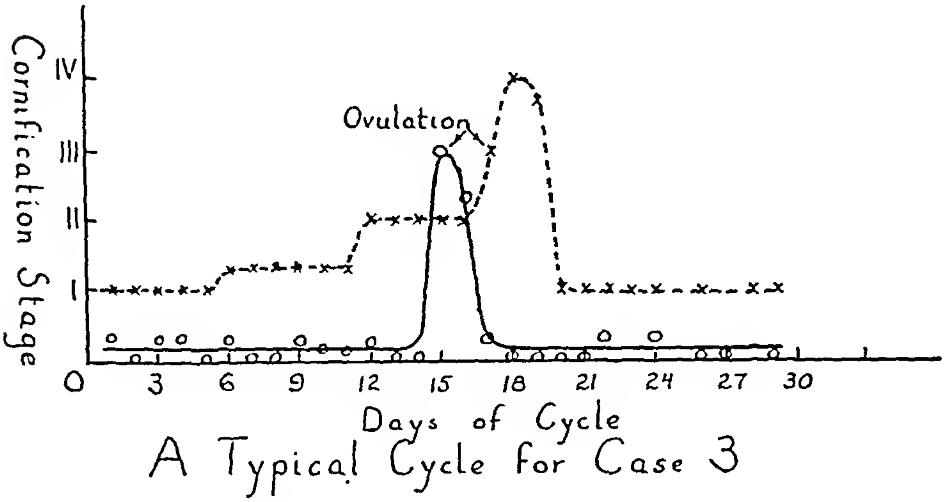
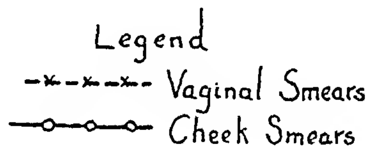


FIG. 24



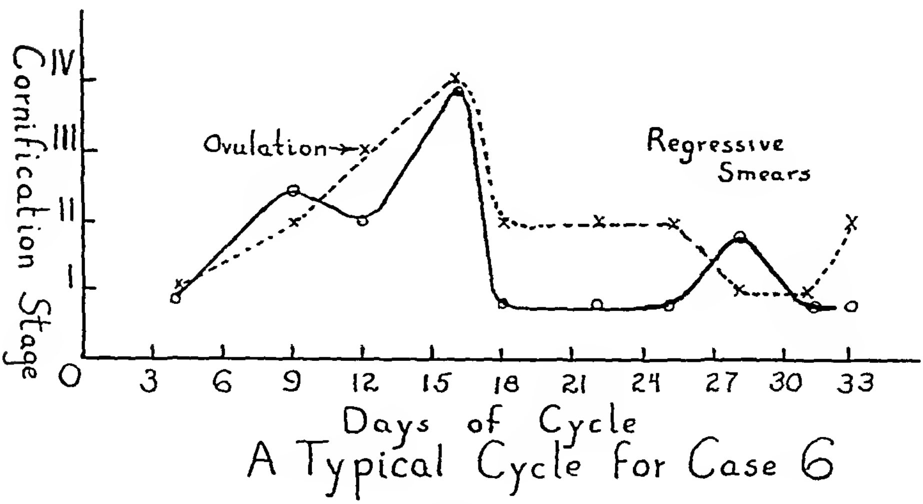


FIG. 25

The cheek smears showed low cornification (about Stage O to I), as in normal women, for six months, after which there was a drop (Stage O). The latter level remained more or less constant during the rest of the observation time. The cheek mucosa, being the least keratinized of the oral mucosae, did not seem to reflect the estrogen changes in this case. However, interesting alterations were seen in the gums. Here cornification was unusually low throughout the entire pregnancy (Stage O-I as compared with Stage III normally). It rose to Stage II ten days after delivery, remained at that level for about 3 weeks, and then fell to Stage I. The gum smears were "dirty" throughout except for the first three days after delivery, when they were "clean." There was an increased tendency to gingival bleeding which persisted until the fifth or sixth postpartum day.

These findings disclose striking similarities in the abnormally low cornification noted in both gum and vaginal smears throughout gestation, and the increased cornification on the ninth day postpartum in the vaginal smear and on the tenth day postpartum in the gum smear. Since the vaginal cornification rose prior to delivery, and the oral did not, the vaginal smear would seem to be a more sensitive indication of the hormonal changes, and would appear to be in agreement with other methods of hormonal assay (19).

Another outstanding difference was the presence of the "oyster" and "navicular" cells in the vaginal smear during gestation, for which no oral counterpart was seen. To our knowledge there is no adequate description in the literature of either oral or vaginal smears during pregnancy with which these observations may be compared.

The following four cases represent observations on the menstrual cycle.

Case 3. Mrs. R., 26 years of age, was studied for three months by means of daily oral and vaginal smears. The patient had a menstrual history of average regularity. Physical examination revealed no abnormalities. One cycle is reported here because the vaginal smears followed the typical pattern described for the normal menstrual cycle (Fig. 24). The peak in estrogen effect on the cheek smears was seen one day before presumptive evidence of ovulation was manifest in the vaginal smear. During the last few days of the cycle the cheek smears were not "clean" and clear-cut as in the beginning. There were many wrinkled, fragmented, anuclear cells which were comparable to the vaginal cells. However, these changes would not have been convincing without the vaginal smears for comparison. The gum smears in this case were unusually "dirty" throughout the three months and little could be learned from them.

Case 4. Miss M., 30 years of age, was studied for six weeks with semi-weekly smears. She had a history of years of irregular menstruation, with periods of from four to eight weeks between menses, but no dysmenorrhea. At one time she had taken an estrogen preparation for this complaint. The vaginal smears showed a change suggesting ovulation, but a full copulative smear was not seen, indicating a possible abnormal estrogen metabolism. The smear of Day 12 of the cycle was of the copulative stage, while that on Day 15 was definitely postovulative. There was more than the usual amount of fragmentation and cellular breakdown before the next menstruation, probably indicating a low hormone level. The gum smears also went through some slight discernible cycle. The highest cornification was on Day 8, preceding ovulation. Cornification was lowest from Day 18 to Day 27, which correlates with the vaginal phase of low hormone production and degeneration.

Case 5. Mrs. H., a 37-year old woman, had oral and vaginal smears taken daily for a month. She had a normal mental, physical, and menstrual history, except that recently she had been experiencing some pain in her breasts in mid-cycle. It was estimated from the vaginal smears that ovulation occurred about Day 12 in one cycle, but the copulative phase was not well developed. There were regressive changes on Days 7 and 8, and also during the last third of the cycle. The change from the copulative to the progestational phase in the gum smear was judged to occur on about Day 10. In the cheek smear there was a drop in cornification after Day 8 as well as an increase in bacteria, debris, and cell fragmentation. The highest cornification was evidenced on Day 9 in the gum smear and three days later in the vaginal smear, thus suggesting a general correlation.

Case 6. Mrs. A., 31 years of age, was studied psychiatrically for 18 months. Oral and vaginal smears were taken simultaneously for two months (Fig. 25). Changes in the vaginal epithelium were comparatively easy to follow and the ovulatory peak was fairly well defined. The cheek smears showed a peak of cornification and increased "cleanliness" during a four-day period which corresponded in time to ovulation, according to the vaginal smears. The progestational phase began simultaneously in both series of smears.

Cases 7 to 16, inclusive, comprise ten middle-aged women whose chief complaint wa either "burning" of the tongue (glossodynia), "burning" of the oral mucosae, or "dryness" of the mouth or throat. The usual causes listed for these complaints, such as pernicious anemia, achlorhydria, etc., were ruled out by physical examination.

The patients were accepted in the order of their appearance at the clinic, no attempt being made at selection. All were past the menopause and in the fifth decade of life. They were given an estrogenic substance: diethylstilbestrol, benzestrol,[3] or estradiol benzoate. In those instances where more than one drug was administered, the medications were given at successive stages of the treatment, not simultaneously. Generally, the dosages were as follows: Diethylstilbestrol, 1 mg. per day, orally; benzestrol, 2 mg. per day, orally; estradiol benzoate, 10,000 R.U. a week, parenterally (Table 2). Oral and vaginal epithelial smears were taken at weekly intervals, or more frequently, and therapy was extended over a period of from two to three months or longer.

The vaginal smears in this experimental series were of the atrophic variety. The gingival smears showed a reduction in keratinization when compared with those of normal young women. Whereas a Stage III keratinization is characteristic of firm, healthy gingivae, both the experimental cases (7 to 16) and control groups (cases 17 to 23) showed low gingival keratinization (Stages I to II). The gross appearance of the gingivae varied little in the two groups.

In all the treated cases (7 to 16) there was evidence of adequate vaginal response to the estrogen, with marked increase in cornification. Seven of the gingival smears in this group revealed an increase in cornification; three showed none. The cheek smears gave evidence of increased cornification in 7 cases, but the rise did not generally coincide with the vaginal response (it came from two to four weeks later) and was less intense. In both sets of smears, those patients receiving the diethylstilbestrol gave the best results whereas the change in the cheek smears was minimal or absent in those treated with benzestrol or estradiol benzoate.

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[3] Product of Research Laboratories of Schieffelin & Company.

CONCLUSIONS

From these studies it may be concluded that:

1) The oral and vaginal epitheliums show parallel changes in degree of cornification during the menstrual cycle, and during estrogenic treatment in deprivation states.

2) Slight hormonal changes are more readily discernible in the vaginal smear than in the oral smear. Consequently, cyclic hormonal changes as seen in the normal menstrual cycle may be better evaluated by means of the vaginal smear.

3) Estrogen deprivation states produce low cornification in both sets of smears, while the response to estrogenic treatment is more strikingly demonstrated in the vaginal than in the oral smear.

4) The vaginal smear, while not an infallible criterion, is a better indicator of ovulation time than the oral smear.

5) The oral smear, despite its technical advantages, cannot replace the vaginal smear as a method of determining hormone levels in women.

ACKNOWLEDGMENT

We wish to express our thanks to Doctors George E. Daniels and S. N. Rosenstein for their assistance in furthering this study. We are also indebted to the Human Sex Biology Gift of Columbia University for contributions toward the compilation of these data.

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CHEMICAL ASSAY FOR "CORTIN"

DETERMINATION OF FORMALDEHYDE LIBERATED ON OXIDATION WITH PERIODIC ACID

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SEVERAL properties of adrenal cortical hormones have been utilized for their assay. The more common bio-assays have depended upon the ability of urinary extracts in adrenalectomized rats or mice a) to maintain life (1), b) to protect against the effects of cold (2), and c) to promote the deposition of glycogen in the livers of fasting animals (3). Because these methods are tedious and prone to considerable biological variation, efforts have been made to develop chemical assays.

The reducing power of corticosteroids has been made the basis for two chemical assays. One procedure employs relatively crude (4), and the other more purified ketonic urinary extracts (5).

The use of the periodic acid oxidation reaction for the estimation of certain urinary steroids was proposed by Fieser, Fields, and Lieberman (6). Under certain conditions the steroid remnant after oxidation is a 17-ketosteroid which can be measured by the Zimmermann reaction. Talbot et al. have used this reaction for the measurement of certain non-ketonic urinary steroids (7). However, the measurement of newly formed 17-ketosteroids has not proved a reliable method for the estimation of urinary corticosteroids (8).

Lowenstein and his coworkers (9) have proposed a method based on the quantitative measurement of the formaldehyde liberated by the oxidation of urinary extracts with periodic acid. In attempting to repeat the detailed procedure supplied to us by the authors, we encountered considerable difficulty. Reasonably reliable results were obtained with crystalline steroids. However, substances interfering with the color reaction were present in small amounts in commercial adrenal cortical extract and in large amounts in many urinary extracts. Because of this difficulty we have introduced a distillation step whereby the formaldehyde liberated by the oxidation is distilled into a sulfite solution leaving behind most of the interfering substances. When the reaction with chromotropic acid is applied to the dis-

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tillate, the color formed is comparable to that obtained with known formaldehyde solutions. The use of this reagent for the determination of formaldehyde in biological fluids was described by MacFadyen (10).

REAGENTS

1. Chloroform, CP.
2. Benzene, CP.
3. Sodium sulfate, anhydrous, CP.
4. Periodic acid reagent: 0.01 M. potassium periodate,[1] in 0.15 M. sulfuric acid.
5. Stannous chloride reagent: Dissolve 6 Gm. of stannous chloride in 10 cc. of hot concentrated hydrochloric acid, dilute with water to a volume of 100 cc. Add a small amount of tin shot to stabilize the solution. Discard when turbid.
6. Chromotropic acid stock solution: 5 per cent chromotropic acid.[2]
7. Chromotropic acid reagent: 4 cc. of chromotropic acid stock solution diluted to 100 cc. with 15 M. sulfuric acid. Make up fresh for every run.
8. Alcoholic sulfite solution: 4 Gm. of sodium sulfite, CP, dissolved in 100 cc. of 1 per cent ethyl alcohol.

METHOD

1. **Extraction:** A 24-hour urine sample is collected with 10 cc. of chloroform as a preservative. The pH of a 200 cc. aliquot is adjusted to about 1.7 with 5 M. sulfuric acid using an indicator paper.[3] Extraction with 200 cc. of chloroform is carried out immediately by shaking in a separatory funnel for five minutes. The emulsion formed is broken by centrifugation and the chloroform layer is drawn off with suction. The remaining urine is re-extracted with 150 cc. and 50 cc. of chloroform successively in a similar fashion. The chloroform extracts are combined and dried over 40 Gm. of anhydrous sodium sulfate for 20 minutes. The solution is then filtered through fluted paper and the sodium sulfate is washed three times with 15 cc. of chloroform.

The extract is next evaporated to a residue (Fig. 1, Residue A) under reduced pressure and a temperature of about 40°C. Residue A is taken up in 10 cc. of benzene and filtered into a 50 cc. florence flask. The original flask is washed twice with small amounts of benzene which are also filtered.

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[1] Obtained from G. Frederick Smith Chemical Co., Columbus, Ohio.

[2] Obtained from Eastman Kodak Co., Rochester, New York. (1,8-dihyroxynaphthalene, 3,6 disulfonic acid, practical.)

[3] "Accutint" paper #20, Anachemia Ltd., Montreal, Canada.

The benzene extract is evaporated to dryness under reduced pressure at about 40°C. to form a residue (Fig. 1, Residue B). It is now possible to take up Residue B in 1 cc. of benzene and transfer it quantitatively to a small centrifuge separatory funnel[4] using two washings of 0.5 cc. of benzene. The final benzene extract with a volume of 2 cc. is partitioned with 5 cc. of distilled water four times, centrifuging at a slow speed each time to

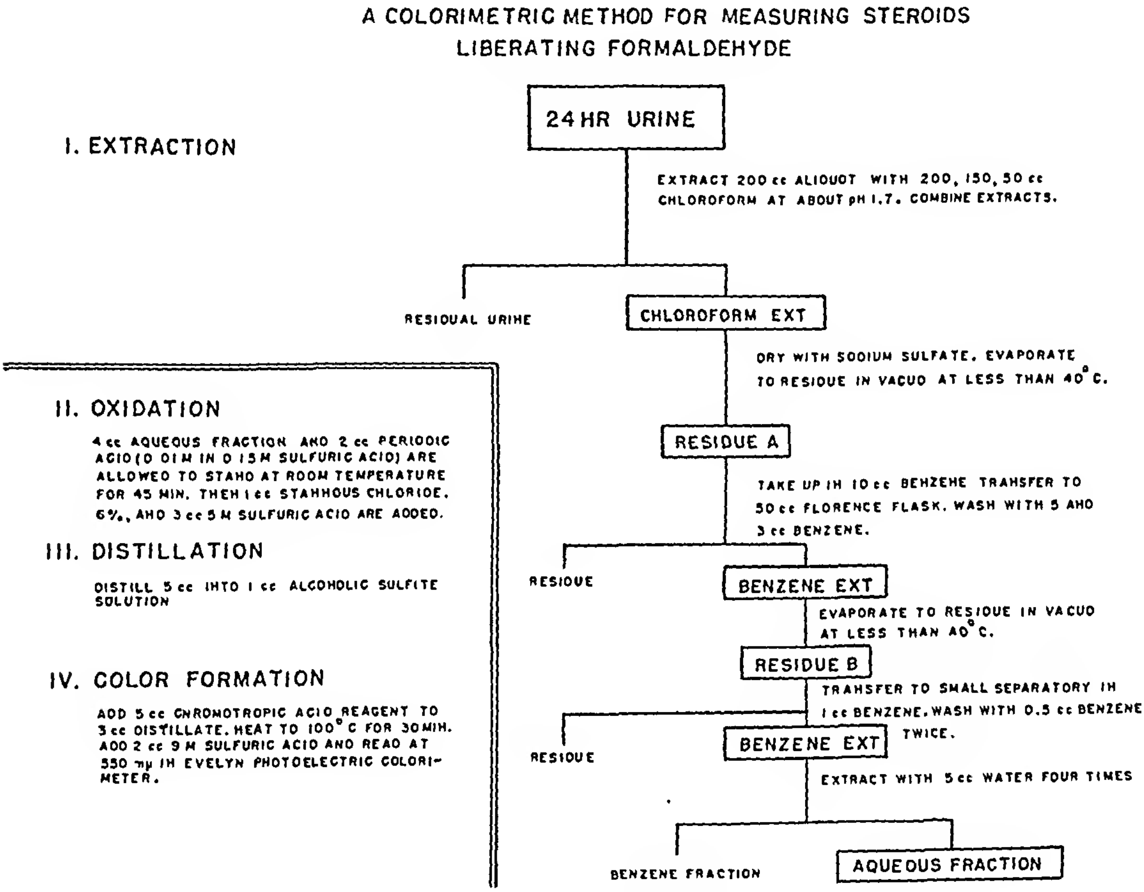

FIG. 1. The extraction and assay procedure in outline form.

break any emulsion. The combined aqueous fraction has a volume of 20 cc.

2. **Oxidation**: Two cc. of periodic acid solution are added to 4 cc. of aqueous fraction and also to 4 cc. of distilled water which is carried through the assay as a blank. Oxidation is allowed to proceed at room temperature for 45 minutes. Excess oxidant is removed by the addition of 1 cc. of stannous chloride solution, forming a white precipitate.

3. **Distillation**: The oxidation mixture is transferred quantitatively to a small distilling flask[5] with three 1 cc. washings of 5 M. sulfuric acid, bring-

[4] A heavy glass separatory funnel of 15 cc. capacity designed to fit a centrifuge cup made by Macalaster Bicknell Co., Cambridge, Mass.

[5] We have used a small all glass distillation apparatus which may be obtained from Macalaster Bicknell Co., Cambridge, Mass.

ing the total volume in the flask to 10 cc. Distillation is carried out by heating with a micro burner until slightly less than 50 per cent of the solution has been carried over. The distillate is collected in a 15 cc. graduated centrifuge tube to which is added 1 cc. of sulfite solution. The tip of the condenser is placed beneath the surface of the solution to prevent loss. The total volume in the receiving tube is adjusted to 6 cc. by the addition of distilled water.

4. Color formation: Five cc. of chromotropic acid reagent are added to 3 cc. of distillate in an Evelyn colorimeter tube. The tubes are heated to 100° C. in a water bath for 30 minutes and then cooled to room temperature. The volume of solution is brought up to 10 cc. by the addition of 2 cc. of 9 M. sulfuric acid and the optical density is measured in an Evelyn photoelectric colorimeter. The galvanometer is adjusted to 100 per cent transmission with the 550 mu filter, using the blank carried through the oxidative procedure mentioned above.

5. Calculations: On a weight basis the amount of formaldehyde formed by the oxidation with periodic acid should be approximately between 8 and 10 per cent of the amount of steroid present, assuming that one mole of steroid liberates one mole of formaldehyde. The conversion factor which we have used in our calculations represents a 7.6 per cent liberation of formaldehyde. In view of the indeterminate nature of urinary corticosteroids it is felt that this approximation is sufficiently accurate for the present. After the isolation and identification of the compounds from the urine it may be possible to select a more accurate standard. The term "cortin" has been retained because of historical precedent, although "formaldehyde liberating compounds" would be more accurate.

Thus:

$$\text{Cortin, mg./day} = \frac{(D)\ (K)\ (10)\ (V)}{200}$$

(D) = optical density = 2 − log G
(K) = conversion factor of optical density to milligrams of cortin = 0.158.
(10) = dilution factor of method
(V) = total volume of 24 hour urine
(200) = aliquot of urine extracted

RESULTS

The extraction procedure we have used conforms in principle to that of Venning, Kazmin and Bell (11) as modified by Lowenstein (12). The extraction is not exhaustive and no doubt some losses occur, but a uniform technique has been utilized throughout so that the relative differences ob-

served probably are significant. It has been demonstrated that acidification results in almost a twofold increase in the amount of biologically active corticoids (8, 11). We have extracted aliquots of the same urine sample at three different pH values and the results are presented in Table 1. It is evident that considerably increased amounts of formaldehyde liberating compounds may be extracted at lower pH values. Heard, Sobel and Venning (4) have demonstrated a similar increase in urinary steroids when measured by their reducing power. The differences in the results obtained by us and Talbot et al. (5) may be explained by the fact that the latter investigators used unacidified urine and used Girard's ketone reagent for purification of the extract. The reason why pH affects the yield of urinary cortin remains obscure. It has been suggested that a labile conjugate of the corticosteroids may be susceptible to such mild hydrolysis (13). The use of strenuous hydrolysis, i.e. concentrated hydrochloric acid and boiling for 7 minutes as carried out for the extraction of 17-ketosteroids, destroys most of the formaldehyde forming substances. This agrees with the observations made using different methods of assay (2, 4).

TABLE 1: THE EFFECT OF pH OF EXTRACTION ON THE RECOVERY OF FORMALDEHYDE LIBERATING COMPOUNDS

Aliquot #	pH	Cortin, mg./day
1	1.7	1.2
2	1.7	1.0
3	3.5	0.75
4	5.4	0.65

The extract has not been washed with water and dilute alkali to remove estrogenic substances and other phenols and pigments, since estrogens do not liberate formaldehyde with periodic acid and urinary pigments do not enter the distillate and therefore do not complicate the procedure.

It is believed that the partition between water and benzene increases the specificity of the method since the property of successive solubility in chloroform, benzene and water is not shared by many compounds capable of liberating formaldehyde. The volume relations of our partition resemble those of Talbot, Saltzman, Wixom and Wolfe (5). These authors have investigated the behavior of five crystalline corticosteroids in such a partition. 17-Hydroxycorticosterone and 17-hydroxydehydrocorticosterone passed quantitatively into the water. Corticosterone and dehydrocorticosterone entered the water only to the extent of 38 and 24 per cent respectively. Desoxycorticosterone remained almost exclusively in the benzene. From these data it appears that water extracts the more oxygenated active corticosteroids from the benzene while compounds with four

oxygen atoms are incompletely removed and those with three oxygen atoms remain almost exclusively in the benzene. Solubility in water is also determined to a large extent by substituents at the 3 position and saturation or unsaturation at the 4–5 position. The partition is not specific for 11-oxy compounds because Δ^4-pregnene-17:20:21-triol-3-one partially enters the aqueous phase (4). We have confirmed the above observations in respect to 11-desoxycorticosterone and the aforementioned pregnene-triol.

Because the partition step represents a potential loss of material we have been interested in the amount of formaldehyde liberating compounds which remain in the benzene fraction. Both the benzene and aqueous fractions of five urinary extracts were assayed and the results are presented in Table 2. In determining the formaldehyde liberating compounds in the benzene fraction we have evaporated off the benzene and dissolved the residue in 1 cc. of ethyl alcohol and then diluted to 6 or 10 cc. with distilled water. The alcohol gives a slight color with the assay and it is necessary to allow for this error. From the results it is obvious that most of the formaldehyde forming compounds in these urinary extracts entered the aqueous fraction.

TABLE 2. RECOVERY OF FORMALDEHYDE LIBERATING COMPOUNDS FROM AQUEOUS AND BENZENE FRACTIONS OF URINARY EXTRACTS

Urine Specimen #	Aqueous Fraction	Benzene Fraction	Per Cent Extracted
	mg./day	mg./day	
82	0.9	Neg.	100
98	0.9	0.08	92
97	1.3	0.26	83
99	1.6	0.19	90
84	1.8	0.27	87

Unfortunately, compounds have not been available for a thorough study of the oxidation reaction with periodic acid. Fieser, Fields, and Lieberman (6) have stated that compounds with a ketol side chain will yield one mole of formaldehyde for each mole of steroid. With a dihydroxyacetone type of side chain the reaction under certain conditions proceeds to form between one and two moles of formaldehyde. With the glycerol type of side chain there is a greater likelihood of the removal of two carbon atoms. With the method we used desoxycorticosterone was found to liberate one mole of formaldehyde. This variability of formaldehyde formation introduces doubt as to the absolute, but not relative, accuracy of the method. It is doubtful if chemical methods measuring the reducing power of these compounds possess any intrinsically greater accuracy because considerable variation exists in reducing power (13).

TABLE 3. EFFECT OF TIME OF OXIDATION ON RECOVERY OF FORMALDEHYDE FROM ALIQUOTS OF AQUEOUS FRACTION

Amount Oxidized (cc.)	Time (min.)	Formaldehyde (micrograms)
4	15	2.4
4	30	2.56
4	45	2.72
4	60	2.72
4	165	3.04

The time of oxidation has been arbitrarily established at 45 minutes. In Table 3 the representative results obtained by oxidation for varying periods of time is given. The reaction proceeds rapidly for about 30 minutes, followed by a gradual progression.

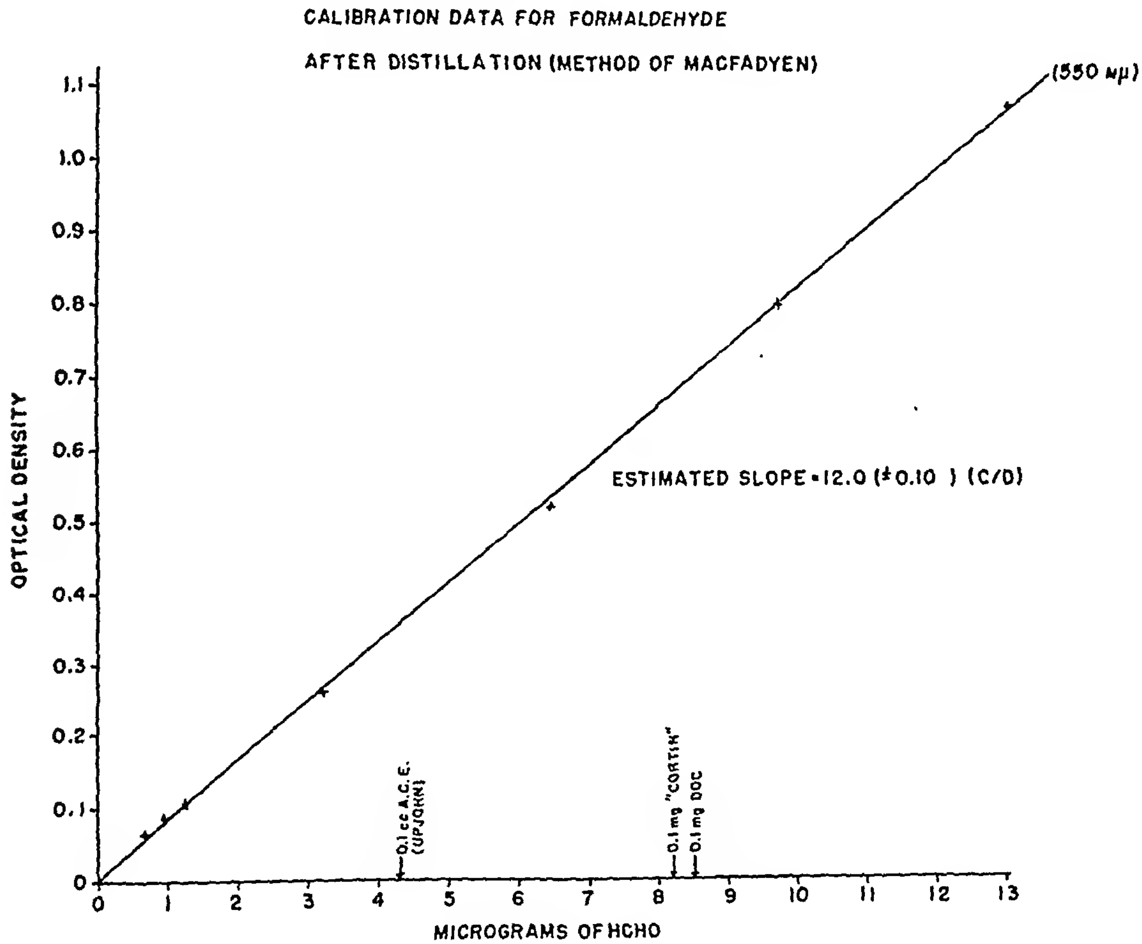

FIG. 2. Hexamethylenetetramine has been used as the source of formaldehyde. The amount of formaldehyde recovered in 3 cc. of distillate is plotted against optical density. The formaldehyde equivalent of 0.1 mg. of 11-desoxycorticosterone, and 0.1 cc. of adrenal cortical extract are compared to 0.1 mg. of our cortin unit.

TABLE 4. EXCRETION OF CORTICOSTEROIDS BY NORMAL SUBJECTS AS MEASURED BY DIFFERENT METHODS OF ASSAY

	Range mg./day	Reference standard
I. Bio-assay		
1. Cold test		
Shipley, Dorfman, Buchwald and Ross (15)	0.5–1.8	11-dehydrocorticosterone
2. Glycogen deposition	0.029–0.055	
Venning et al. (3)	males	
	0.045–0.060	11-dehydro-17-hydroxy-
	females	corticosterone
II. Chemical Assay		
1. Reducing power		
a. Talbot et al. (5)	0.12–0.34	Corticosterone
b. Salter et al. (16) (modification of II a)	0.14–0.67 males	?
c. Heard, Venning and Sobel (4)	1.1–2.1	
	males	
	1.0–2.0	
	females	11-desoxycorticosterone
2. Formaldehyde liberation		
Authors' method	1.0–1.6	Cortin unit, see text

The distillation step separates substances present in urinary extract following periodic oxidation, which modifiy the color reaction. Figure 2 presents the calibration data for our apparatus employing the method of MacFadyen using hexamethylenetetramine as the source of formaldehyde. A linear relation exists over the range of concentration encountered in this procedure, although all of the formaldehyde in the distillate is not recovered.

Results of 120 determinations made on 70 patients and normal subjects are to be presented in a separate communication. The limits of normal have not been established with any finality but it is evident that under the usual conditions of diet and activity the excretion of cortin is between 1.0 and 1.6 mg. a day. The excretion of this material is correlated with known disorders of adrenal function. The normal values obtained by other chemical methods are presented in Table 4.

CONCLUSIONS

A method has been described which can be used satisfactorily for the quantitative estimation of urinary corticosteroids. The procedure is sufficiently simple to be useful in clinical as well as in research studies.

The extraction is carried out on acidified urine with chloroform. A partition between benzene and water is used and the aqueous fraction thus formed is oxidized with periodic acid. The formaldehyde formed by this reaction is distilled from interfering chromogens and the amount of color formed with chromotropic acid is measured.

The results that have been obtained correspond in order of magnitude to other chemical methods applied to acidified urine.

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HORMONE SECRETION BY HUMAN PLACENTA GROWN IN TISSUE CULTURE

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IS THE placenta an organ of internal secretion producing gonadotropins and estrogens during pregnancy? This question has been propounded since 1927 when these hormones were first demonstrated in large amounts in the blood and urine of pregnant women. Many workers have demonstrated the presence of gonadotropins and estrogens in extracts of human placental tissue and it has therefore been accepted that the placenta is an organ of internal secretion and the source of these hormones. Articles bearing on this subject have been voluminous in their theoretical views but have been inconclusive due to lack of direct reasoning and rigid proof.

Renewed interest in the subject has brought forth direct evidence of the secretory function of the placenta. The tissue culture studies of Gey, Seegar, Jones and Hellman, (1, 2) as well as the histochemical presentations of Wislocki, Dempsey and Bennett, (3, 4) and Baker, Hook and Severinghaus (5) are recent and valuable contributions in this field of endocrinology. Our study is presented as further direct evidence of the secretion of the gonadotropins and estrogens by human placental tissue grown in tissue culture.

METHOD OF TISSUE CULTURE

Placental tissue was collected under sterile conditions in the operating room from patients requiring therapeutic termination of pregnancy. The first specimen for tissue culture was obtained by the transvaginal route by cervical dilatation and evacuation of the uterine cavity while in all other cases the abdominal hysterotomy route was used.

Normal appearing placental fragments were selected and placed in a sterile Petri dish with some clotted blood to keep the fragments moist. The tissues were transferred immediately to the tissue culture laboratory.

The tissue culture method used was that originated by Löwenstädt, (6) as suggested to him by Carrel and later revised by Gey (7) with subsequent modifications by Lewis (8). Fragments of placental tissue measuring 2 mm. or slightly less in size were rapidly washed in Tyrode's solution

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* This study was begun in 1941 by the authors at Temple University Hospital and Medical School, and completed by H. L. Stewart, Jr. at the Henry Ford Hospital.

(pH 7.6–7.8). Twenty-eight fragments were implanted in each of four pyrex tubes, 15 × 1.4 cm. The drum in the incubator rotated at a constant speed of one turn in 20 minutes. The incubator was automatically regulated at a temperature of 37.3 degrees centigrade. Several pieces of identical character were selected for histological study. The culture medium consisted of 0.15 ml. of Tyrode's solution, 0.15 ml. of embryonic mouse extract and 0.15 ml. of heparinized chicken plasma (animals less than one year of age). Identical control tubes using mouse spleen or lung tissue were inoculated and at regular intervals this medium was subjected to endocrine assay. The tissue culture procedure was done at room temperature under careful aseptic technique and all solutions and extracts were tested for bacterial contamination. Cell growth was studied under low power in the tubes, while more detailed observations under high power and oil immersion were made in accordance with the Sano-Smith technique (9). At intervals varying from three to seven days, 1.2 cc. of Tyrode's solution was added to the tubes. The tubes were rinsed with this solution by rotating it manually. All washings from these tissue culture tubes were collected and sent to the Laboratory of Endocrinology. As individual pipettes were always used for each step and each solution, the possibility of error and contamination was practically eliminated.

METHOD OF ENDOCRINE ASSAYS

In three placental tissue culture studies, preoperative serum gonadotropin and estrogen assays were made on the blood of the placental donor. On the day of termination of the pregnancy, 50 cc. of blood were obtained by venopuncture. The blood was permitted to clot in a large test tube and was sent directly to the Laboratory of Endocrinology where the clot was broken up and the specimen centrifuged. The serum was used for biological determination of the gonadotropins and estrogens.

Tissue culture washings and tissue culture control specimens were sent directly to the Laboratory of Endocrinology from the Tissue Culture Laboratory. The exact contents of the control specimen were unknown to those doing the hormone titrations until the assays were completed.

All specimens were kept in the icebox without the addition of any preservative and animal injections were begun on the day that the specimens were received. Previous curves for the serum gonadotropins and estrogens during pregnancy had been established in our laboratory on normal pregnant subjects. Suitable dilutions of the sera were made in accordance with the duration of the pregnancy at the time of termination of the gestation. Distilled water was used as the diluent for both sera and tissue culture fluids.

GONADOTROPINS

Adult female isolated rabbits and infantile female mice were used for determinations of the gonadotropin content of the blood and tissue culture washings.

In the standardization of our laboratory animals, we had correlated the relationship of the rabbit to the mouse unit and had found that approximately two and a half mouse units were equivalent to one rabbit unit. This observation permitted us to make a preliminary rapid assay of the gonadotropin content and thereby obtain the approximate amount of hormone within a 48 hour period. Adult female rabbits weighing from 1800 to 2000 grams which had been isolated for 30 days were used. Two rabbits were injected at each dosage level and suitable dilutions of the sera or culture fluid injected over a wide quantitative range. The diluted fluid was injected intravenously in one injection. Forty-eight hours later the ovaries were examined grossly by laparotomy under ether anesthesia. Any ovary showing formation of corpus hemorrhagicum or corpus luteum in one ovary was considered positive. Ovarian sections were made if doubt existed in any case as to the presence of a corpus hemorrhagicum or corpus luteum.

Knowing the approximate titre of the gonadotropins from the preliminary rapid quantitative rabbit determination, we then proceeded with the quantitative assay using infantile female mice, 22 to 25 days of age and weighing from 8 to 10 grams. Injections were made subcutaneously each morning for three days and on the morning of the fifth day the animals were killed by gas asphyxiation. Four to six animals were used at each dosage level.

A final titration was then performed using both infantile female mice and adult isolated female rabbits. As many mice were used as possible with a minimum of 8 to 10 animals for each level. Two rabbits were injected for each level. The presence of corpora hemorrhagica or corpora lutea by histological study of the ovarian section in 50 per cent of the mice and rabbits constituted a positive test.

ESTROGENS

The Fluhmann technique (10) was used for determination of the estrogens in the sera and the tissue culture washings. No dilutions of either fluid were necessary for the amount present was either very minimal or negative Adult spayed female mice weighing 25 grams and approximately 8 weeks of age, 8 days postoophorectomy, were injected subcutaneously twice daily for 3 days with 0.75 cc. for a total of 4.5 cc. The animals were killed on the morning of the fourth day and midvaginal biopsies were made. Histological studies of the vaginal biopsies were made and the amount of estrogens estimated in accordance with the Fluhmann technique.

Six animals were used for each blood serum estimation. The amount of tissue culture fluid limited the number of animals which could be used in each assay, but no less than two animals were used for any one determination.

RESULTS

Histological sections of early placental tissue as well as the more mature placenta of eight and nine months revealed in every case the presence of normal tissue free of any abnormal degeneration or abnormal growth.

The control tissue culture washings showed complete absence of any chorionic gonadotropin and estrogen activity in test animals. Control assays were made at intervals during the study of the placental tissue cultures.

Growth of the adult placenta of eight and nine months duration was unsuccessful on several occasions. Study of these tissue cultures revealed overgrowth of fibroblastic tissue and a complete absence of Langhans and syncytial cells. Endocrine assays were negative for the gonadotropins and estrogens.

PLACENTAL TISSUE CULTURE I

This was placental tissue from a pregnancy of five weeks duration. The termination of pregnancy was by the transvaginal route because of maternal pulmonary tuberculosis. The growth was profuse and three types of cells typically seen in our tissue cultures were present. Cells similar to the syncytial cells appeared to multiply though at no time were actual mitoses noted. Degeneration of these cells appeared early and soon rapid disappearance was evident. Fibroblasts grew relatively well but the Langhans cells with their characteristic clear halo around the nucleus grew most profusely. Fig. 1 is an unstained in vitro culture of the growth seven days after inoculation into the medium showing these cell types. After three weeks the growth rapidly diminished and as shown in Table 1 there was a corresponding diminution of the gonadotropin titre.

Preoperative serum gonadotropin and estrogen assays were not made, for at this time we were interested only in determining whether the placental cells would grow and whether they were active in the production of gonadotropins. Finding that the growth was producing this hormone, our attention was then focused toward correlating the type of cell growth present to the exclusion of any estrogen determinations.

PLACENTAL TISSUE CULTURE II

This was placental tissue from a pregnancy of three months duration. As illustrated in Table 2, preoperative serum gonadotropins showed 20,000 to 24,000 mouse units per 100 cc. of blood while the quantitative Friedman test was positive to 1.65 cc. of 1:20 dilution of the serum. Serum

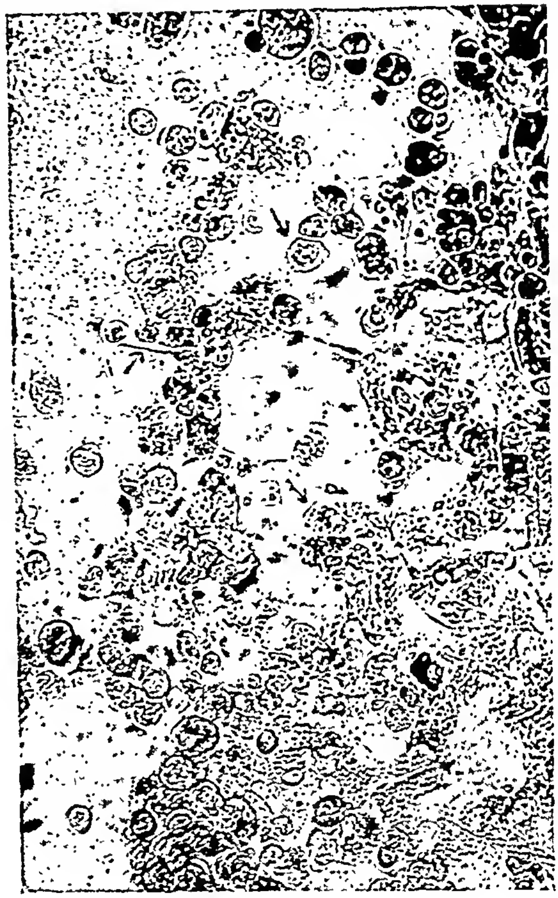

FIG. 1. Unstained tissue culture, seven days after inoculation. ×180. "A"—stromal cell; "B"—syncytium; "C"—Langhans cell.

TABLE 1. PLACENTAL CULTURE I

A.B. Age 35. White. Multipara.
L.M.P. February 1, 1941
C.D.C. November 8, 1941

April 30, 1941—Termination of pregnancy

Quantitative Assay of Tissue Culture Fluid

Specimen	Days Cultured	M.U. per cc. Gonadotropin	Rabbit Gonadotropin	Estrogen
A	7	1.0		
B	14	1.0		
C	19	1.0	No assays	
D	24	0.5		
E	30	negative		

TABLE 2. PLACENTAL TISSUE CULTURE II

T.M. Age 24. White. Multipara.
L.M.P. March 18, 1941.
C.D.C. December 25, 1941.

June 10, 1941—Preoperative serum gonadotropin and estrogen specimen.
Serum gonadotropins:
20,000 to 24,000 M.U. per 100 cc.
Serum estrogens:
6 to 9 M.U. per 100 cc.
Quantitative Friedman Test (serum):
Positive: 1.65 cc. of 1:20 dilution.
Negative: 1.45 cc. of 1:20 dilution.

June 10, 1941—Termination of pregnancy by hysterotomy.

Quantitative Assay of Tissue Culture Fluid

Specimen	Days Cultured	M.U. per cc. Gonadotropin	Rabbit Gonadotropin	Estrogen
A	7	—	—	Negative
B	10	1.5	1.5 cc. positive	—
C	15	1.0	—	Negative
D	20	—	—	Negative
E	27	0.5	—	—
F	31	Follicle stimulation; Uterine growth moderate. No C.H. or C.L.		

estrogen activity showed six to nine mouse units per 100 cc. (Fig. 2). The growth was not particularly good and the Langhans cells visualized early as growing, soon degenerated after two and one-half to three weeks. Hormone assays for the gonadotropins showed a parallel decrease in activity.

PLACENTAL TISSUE CULTURE III

Table 3 shows in graphic form the assays of a placental tissue culture growth of approximately seven weeks duration. Preoperative serum gonadotropins were approximately 3500 mouse units per 100 cc. Serum estrogens were not demonstrable. Injections of 1.35 cc. of 1:40 dilution of the donor serum gave a positive Friedman test.

Growth was profuse and the most abundant we observed in any of our cultures. Numerous well balanced mitoses were observed. Langhans cells predominated throughout and became more abundant as the tissue grew.

Figure 3 shows the Langhans cells with the clear halo around the nucleus and several mitoses. By the Sano-Smith method it is possible to stain these cells and mount the slides without manipulating or disturbing the growth.

Figure 4 portrays the cells at the peak of their growth with evidence of

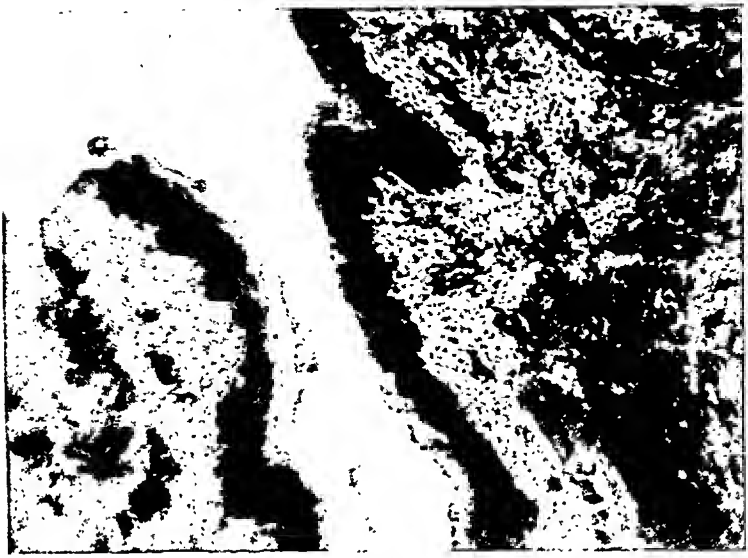

Fig. 2. Vaginal mucosa of mouse showing reaction 3 in the mucification test for estrogen; approximately 6 mouse units per 100 cc. of blood.

degeneration of a few. This photograph was taken one month after they had been inoculated into the medium. At this time there was a rise in the gonadotropin titre. Six weeks later they were still healthy in appearance but not actively growing as noted by the presence of only a rare mitosis.

Fig. 3. Twelve days growth. "A"—syncytium; "B"—Langhans cell. ×300. Toluidine blue stain.

TABLE 3. PLACENTAL CULTURE III

D.B. Age 30. White. Multipara.
L.M.P. August 26, 1941.
C.D.C. June 2, 1942.

October 24, 1941—Preoperative serum gonadotropin and estrogen specimen.
Serum gonadotropins:
3500 M.U. per 100 cc.
Serum estrogens:
None demonstrable.
Quantitative Friedman Test (serum):
Positive: 1.35 cc. of 1:40 dilution.
Negative: 1.20 cc. of 1:40 dilution.

October 24, 1941—Termination of pregnancy.

Quantitative Assay of Tissue Culture Fluid

Specimen	Days Cultured	M.U. per cc. Gonadotropin	Rabbit Gonadotropin	Estrogen
A	5	—	—	Negative
B	10	55	0.5 cc. 1:40	Negative
C	13	50	1.0 cc. 1:40	Negative
D	19	40	1.5 cc. 1:40	—
E	22	—	—	Negative
F	25	22	1.0 cc. 1:20	Negative
G	31	26	1.0 cc. 1:20	—
H	35	—	—	Negative
I	40	20	1.0 cc. 1:20	—
J	45	—	2.0 cc. 1:20	Negative
K	50	5	—	Negative
	Study discontinued.			

In this study repeated estrogen assays were made even though there was an absence of other cell types including syncytial cells. All assays for estrogens were negative and Figure 5 is a typical example of the many negative biopsies of the mouse vaginal mucosa. Difficulty in growth of the syncytial cells was constantly in evidence.

PLACENTAL TISSUE CULTURE IV

Prior to termination of pregnancy, large amounts of gonadotropins were present in the blood of the placental donor (Table 4). The serum estrogen was approximately 12 mouse units per 100 cc.

The tissue culture growth was quite abundant although less than in

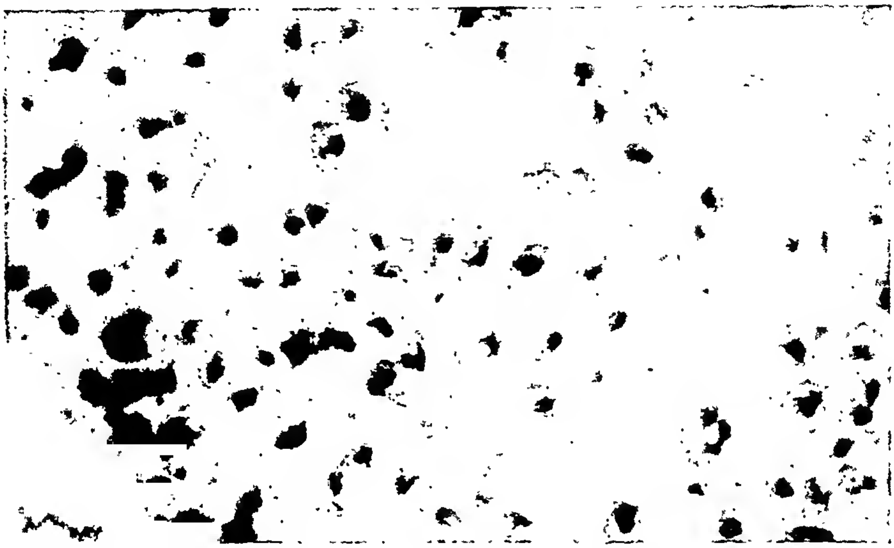

FIG. 4. Peak of hormone production. Some of the Langhans cells are undergoing degeneration. Mostly Langhans cells. ×300. Toluidine blue.

placental Tissue Culture IV. Fewer mitoses were present. The cytoplasm of the Langhans cells was slightly more granular (Fig. 6). The gonadotropin titre was not as high. Degeneration of the cells had appeared to set in about the third week and little hormone production was expected. However, titrations of the washings proved the contrary. The mouse ovaries

FIG. 5. Vaginal mucosa of mouse showing no estrogenic response. Mucosa shows two layers of low cuboidal epithelium.

TABLE 4. PLACENTAL CULTURE IV

D.R. Age 23. White. Multipara.
L.M.P. December 9, 1941.
C.D.C. September 16, 1942.

February 20, 1942—Preoperative serum gonadotropin and estrogen specimen.
Serum gonadotropins:
4000 M.U. per 100 cc.
Serum estrogens:
12 M.U. per 100 cc.
Quantitative Friedman Test (serum):
Positive: 2.25 cc. of 1:40.
Negative 1.50 cc. of 1:40.

February 21, 1942—Termination of pregnancy.

Quantitative Assay of Tissue Culture Fluid

Specimen	Days Cultured	M.U. per cc. Gonadotropin	Rabbit Gonadotropin	Estrogen
A	5	40	2.5 cc. 1:40	
B	10			Negative
C	13	35	3.0 cc. 1:40	Negative
D	18		3.0 cc. 1:20	Negative
E	23	20	2.0 cc. 1:20	
F	28	12	2.0 cc. 1:10	Negative
G	33	10	2.0 cc. 1:10	
H	38	8	3.0 cc. 1:10	Negative
I	44	4	0.5 cc.	Negative
J	48	6	0.5 cc.	
K	55	4	0.5 cc.	
L	61	2	0.5 cc. Negative	
M	70	1	1.0 cc. Negative	
N	75 Follicle stimulation. Uterine growth marked. No C.H. or C.L.		1.0 cc. Negative	

typically showed the presence of corpora lutea and corpora hemorrhagica two months after implantation (Fig. 7). It was believed that the hormone was produced by fragments of tissue deeper in the medium which could be seen with the naked eye but could not be observed under the microscope because of the thickness of the glass and the medium. Figure 8 shows the placental culture at 70 days with marked degeneration of the Langhans cells.

Syncytial cells failed to grow well and all estrogen assays were negative.

DISCUSSION

Our placental tissue culture studies revealed that in the early stages of growth three types of cells could readily identified:—stromal cells with a round nucleus, syncytial cells which are oblong and have an oval nucleus, and Langhans cells which are more or less round and have a round nucleus surrounded by a pale halo (Fig. 1). After a few days of growth the latter cells predominated. Soon a direct relationship was noted between any

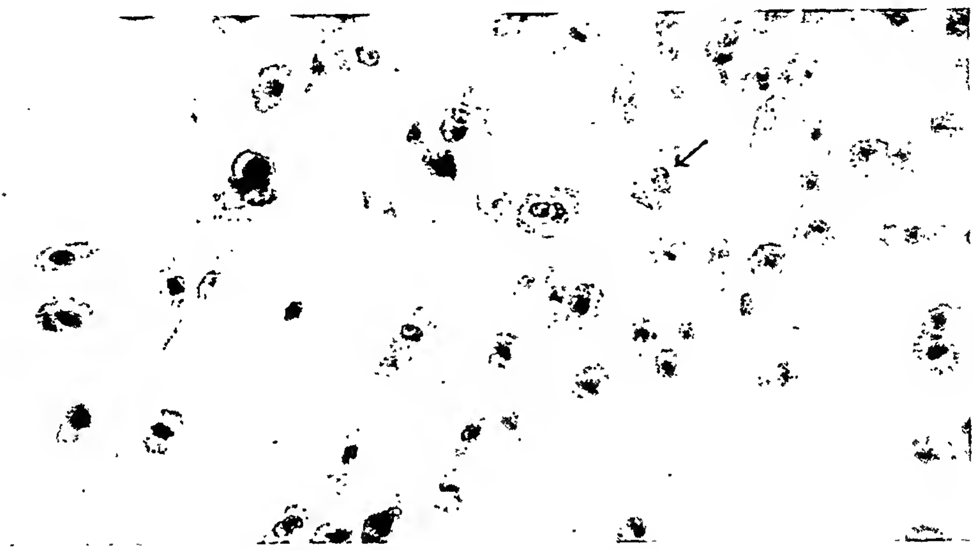

FIG. 6. Four days growth, mostly Langhans cells, in three weeks old placental tissue. Note the clear halo around the nucleus (arrow).

increase or decrease in the hormone titrations for the gonadotropins and the Langhans cell growth. This suggested to us the important role that these cells play in the secretion of the gonadotropic hormone and we believe that they are probably responsible for the great amounts of this hormone production during pregnancy. Baker, Hook and Severinghaus (5) believe that occasional Langhans cells persist beneath the syncytium until term. It is reasonable to assume that the relatively lower level of the blood and urinary levels of gonadotropins in later pregnancy fall in direct correlation with the fewer number of Langhans cells.

Our results are in support of those of Gey and his coworkers (1, 2). They showed that placental cells maintained in continuous culture for as long as six months produced gonadotropins. The cells apparently responsible for this activity were the Langhans cells.

It is interesting to note that our preoperative serum assays for the estrogenic hormone were small in amounts until the third month of pregnancy.

In two cases, 3 and 4, small amounts were present showing some increased production at the time of termination of the pregnancy. Wislocki and Dempsey (3, 4) as well as Baker, Hook and Severinghaus (5) believe that the syncytial cells are the source of estrogen formation in the placenta. In our tissue cultures, syncytial cells were noted in the early growth stages but quickly disappeared as the Langhans cells became the predominant cell to the

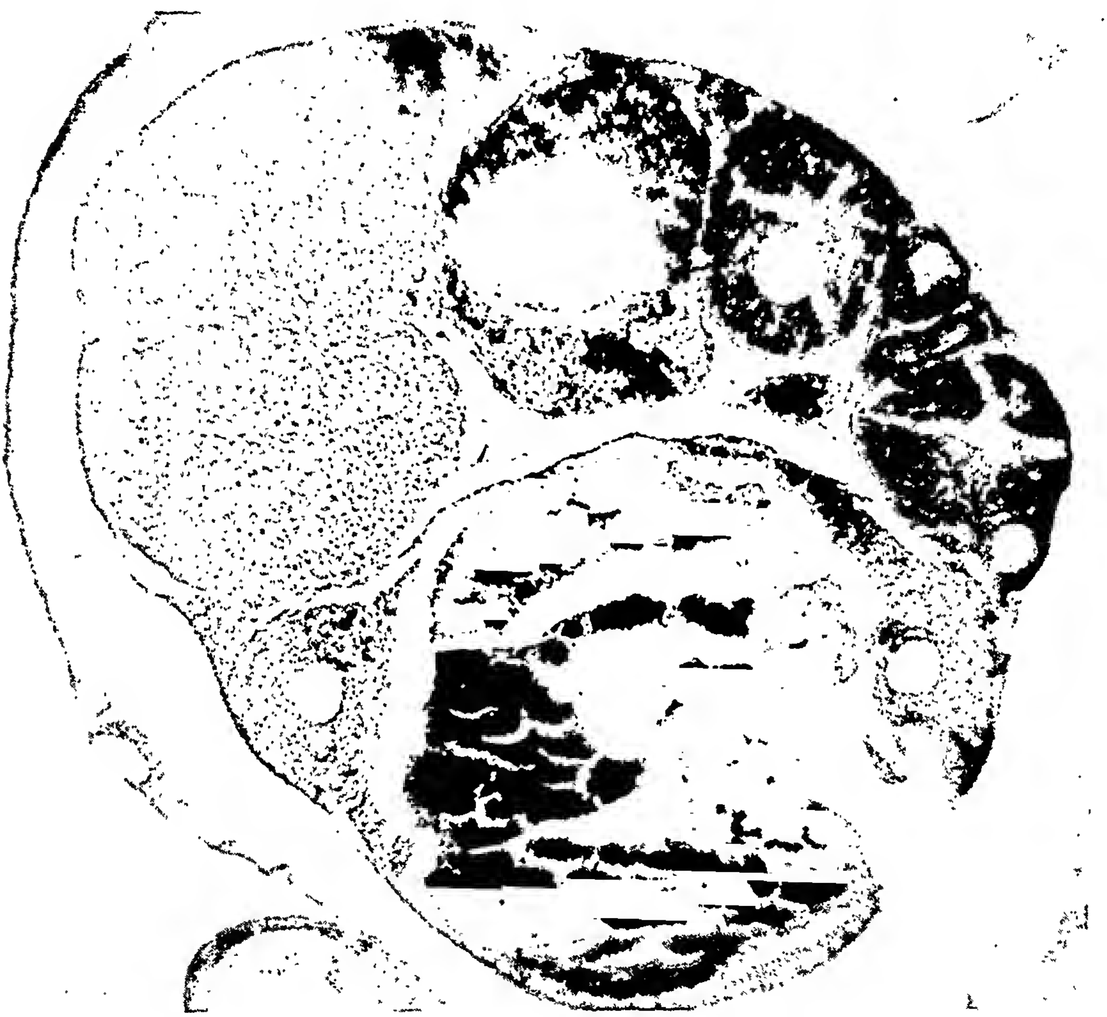

FIG. 7. Infantile mouse ovary showing formation of corpora hemorrhagicum and luteum. Reaction obtained by injection of 0.5 cc. tissue culture washing 60 days after placental tissue implanted.

exclusion of the syncytial cells. In placental tissue culture assays of III and IV, many estrogen determinations were made without any evidence of its presence. Yet our negative results, we believe, are inconclusive and not in favor of or against the secretion of estrogens by the placenta. Since so little increase of estrogen was present in the blood of the patient prior to termination of pregnancy and since the syncytial cells did not grow in tissue

culture, demonstrable amounts could not be expected from our tissue cultures on the basis of the present viewpoint.

Our curves of estrogen in the blood and urine of normal pregnant women show a gradual rise from the third month to the highest peak just prior to parturition. Because of this we attempted on several occasions to grow the more mature placenta of eight and nine months. The growth was un-

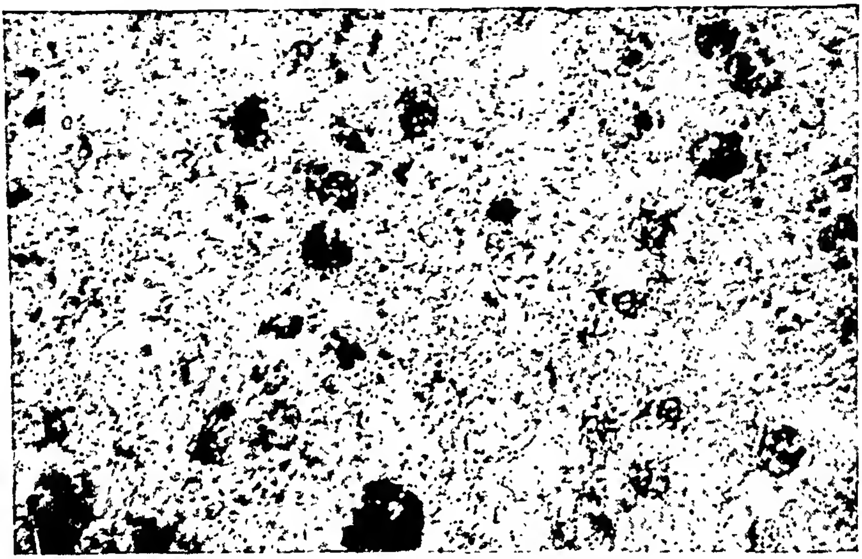

FIG. 8. After peak of hormone production there is marked degeneration of the cells. ×300. Toluidine blue.

successful for fibroblasts grew in profusion without demonstrable syncytial or Langhans cell growth. It is interesting that the trophoblastic tissue of early pregnancy grows so readily in comparison with the more mature or adult placenta.

CONCLUSIONS

1. Human placental cells of approximately three months of age, grown in continuous tissue culture in vitro, showed the production of the gonadotropic hormone as assayed quantitatively in rabbits and infantile mice.
2. A direct correlation was noted between the growth of the Langhans cells and the production of the gonadotropic hormone.
3. Control tissue cultures in which fibroblasts were grown produced no hormone response in the test animals.
4. Attempts to grow the adult placenta of eight and nine months were unsuccessful and resulted only in overgrowth of fibroblastic tissue.

5. Syncytial cells did not grow well in tissue culture.
6. Evidence concerning the production of estrogen by the placental cells was inconclusive.

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ENDOCRINE REVIEWS

There has been a great demand from our members for good review articles in the Journal of Clinical Endocrinology. It was felt that these articles would be of value both to endocrinologists and to physicians in other fields of medicine who wish to keep themselves informed about advances in endocrinology. The excellent review by Dr. Harold L. Mason of the Mayo Clinic on Steroid Nomenclature is the first of a series of such articles. Many of our members have already agreed to prepare reviews for the journal. These will appear from time to time as they become available.

WILLARD O. THOMPSON,
Managing Editor.

STEROID NOMENCLATURE

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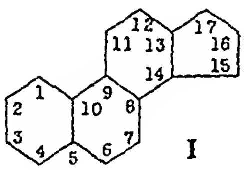
INTRODUCTION

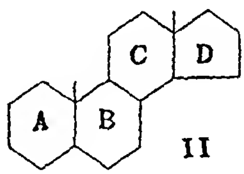
IT IS the purpose of this paper to consider the nomenclature of the steroid hormones and some of their important derivatives. It is intended for those who have occasion to deal with steroids but who do not have an intimate acquaintance with their chemistry or with the principles by which they are named. The complexities of some of the names and lack of agreement among chemists about some details of terminology may well serve to confuse the uninitiated. Almost any compound may be named in several different ways. The chemist often names a compound in a manner that serves to emphasize a certain feature or features of that compound. In this paper the names that are used most commonly in this country will be emphasized and variations in these names will be given. It is purposed only to interpret steroid nomenclature, not to advocate any particular system. However, some preferences will be stressed in the interests of good chemical usage.

THE STEROID RING SYSTEM

The fundamental ring system of the sterols, bile acids, sex hormones and adrenal hormones, the latter three being grouped under the term "steroids"



I



II

FIG. 1

is the perhydrocyclopentenophenanthrene ring system (Fig. 1, I). The system[1] used for numbering the carbon atoms of the steroids is also shown in formula I. This is a saturated ring system.[2] Each juncture of two or

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[1] It should be pointed out that the cyclopenteno ring (the ring with five carbon atoms) may be attached to phenanthrene in several different positions with the formation of a number of different cyclopentenophenanthrenes. Also, the steroids have a unique numbering system which is not related to the system that has been adopted for phenanthrene and its derivatives.

[2] The name "perhydrocyclopentenophenanthrene" may seem to disagree with the

more lines in I and II represents one carbon atom. Each carbon atom, except those common to two rings, bears two hydrogen atoms. Ordinarily these hydrogen atoms are not written in the structural formula. It is understood that those bonds of the carbon atoms which are not shown are combined with hydrogen. A hydrogen atom attached to a carbon atom which is common to two rings may be written in to show the stereochemical structure at this point. The estrogens have a methyl group (CH_3) attached at position 13. Most of the other steroids (II) have methyl groups (indicated by the short vertical lines in II) attached at carbons numbered 10 and 13 (C-10 and C-13). The methyl groups are known as angular methyl groups because they occur in the angle formed by the juncture of two rings.

SUBSTITUTION IN THE STEROID RING SYSTEM

In conformity with the rules of organic nomenclature, many steroids are named as derivatives of a few hydrocarbons such as cholane, etiocholane, pregnane, and so forth. Others, such as androsterone and progesterone, have received trivial[3] names which are commonly used. The ending "-ane" is reserved for saturated hydrocarbons. The ending "-ene" indicates a hydrocarbon with a double bond; "-diene," "-triene," "-tetraene" indicate the presence of two, three, four double bonds, and so on. Substitution of oxygen for hydrogen in the hydrocarbon is shown by addition of "-ol" or "-one" to the name of the hydrocarbon. The suffix "-ol" represents an alcohol group (hydroxyl or OH) and "-one," a ketone group (C=O, also called a carbonyl group). Examples: pregnane, pregnanol, pregnanone (the final *e* of "-ane" and "-ene" is dropped when followed by another vowel), pregnanediol, pregnanedione; pregnene, pregnenol, pregnenediol. Alternatively these substituents may be indicated by the prefixes "hydroxy-" and "keto-" (or "oxo-") respectively. Pregnanol would thus be hydroxypregnane; pregnanone would be ketopregnane (or oxopregnane), and pregnanediol would be dihydroxypregnane. These prefixes and suffixes, and some others, and their meanings, are listed in Table 1 for convenient reference.

If a compound contains both hydroxyl (alcohol) and ketone groups the order in which the prefixes or suffixes appear in the name is largely a matter of choice. Usually the one that indicates a hydroxyl group (or groups)

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naming of saturated hydrocarbons which is discussed in the next paragraph. However, the prefix "perhydro-" means that cyclopentenophenanthrene (an unsaturated hydrocarbon) has been saturated with hydrogen. There is no simple name ending in "-ane" for this saturated hydrocarbon. In a similar manner the saturated hydrocarbon ethane could be named "dihydroethylene."

[3] "Trivial" is used in the sense of common or ordinary.

TABLE 1. MEANINGS OF SUFFIXES AND PREFIXES USED IN NAMING THE STEROID HORMONES

Suffix	Meaning
-ane	Saturated hydrocarbon. Cannot take up more hydrogen
-ene	Unsaturated hydrocarbon; has double bonds. Can take up more hydrogen
-ol	Alcohol; hydroxyl group; OH
-one	Ketone; carbonyl group: C=O
Prefix	
hydroxy-	Hydroxyl group; OH
keto-	Ketone; carbonyl group; C=O
oxo-	Same as "keto"
allo-	Other; meaning one of two isomers
cis-	Refers to arrangement of two groups on the same side of a molecule
trans-	Refers to arrangement of two groups, one of which is on the opposite side of a molecule from the other
etio-	Refers to the final degradation product of a more complex molecule which still retains the essential chemical character of the original molecule
iso-	Refers to isomers
epi-	Refers to isomers which differ only in the spatial arrangement of groups about one carbon atom
desoxy-	An oxygen atom has been lost
dehydro-	Two atoms of hydrogen have been removed

precedes the one that indicates a ketone group (or groups). Thus "pregnanolone" is preferred to "pregnanonol" and in the alternative method of naming, "hydroxyketopregnane" ("hydroxyoxopregnane") is preferred to "ketohydroxypregnane."

Each carbon atom of a hydrocarbon is assigned a number. When a hydrogen atom (or atoms) attached to a carbon atom is substituted by another

atom or group of atoms, the number of that carbon atom is placed before the prefix or suffix which designates the nature of the substituent. Thus, pregnan-3-ol or 3-hydroxypregnane; pregnane-3,20-diol or 3,20-dihydroxypregnane. In some of the literature the numbers follow the substituents, as in pregnanediol-3,20, pregnanol-3-one-20. However, it is preferable to follow the usage of Chemical Abstracts and the Journal of the American Chemical Society and to place the number before the substituent.

Some authors arrange the suffixes and their numbers in such order that the lowest number comes first. They would write "pregnan-3-ol-20-one" but "pregnan-3-on-20-ol" instead of "pregnan-20-ol-3-one," and "11-dehydro-17-hydroxycorticosterone" rather than "17-hydroxy-11-dehydrocorticosterone." This method is used by Selye (1).

DOUBLE BONDS

A double bond and its position are often indicated by the symbol Δ used as a prefix and with a superscript which shows the position of the double bond. Examples: Δ^{5}-androstene, Δ^{4}-pregnene-3,20-dione. It is understood that the bond originates at the carbon atom shown by the superscript, and terminates at the carbon atom with the next higher number. If there is any doubt, numbers are given for both ends of the double bond. For example, a bond originating at C-8 may terminate at C-9 or at C-14. In this instance the position of the double bond is shown by $\Delta^{8,9}$ or $\Delta^{8,14}$ as the case may be. Use of Δ^{8} would be ambiguous. The suffix "-ene" is always used in addition to Δ. If more than one double bond is present the positions of both are shown and two double bonds are indicated in the name. Examples: $\Delta^{3,5}$-androstadiene-17-one, $\Delta^{5,16}$-pregnadiene. Multiple double bonds are shown in similar fashion. Although it might appear that some ambiguity would result from the use of two numbers in the superscript to indicate one or two double bonds, a brief consideration of the numbers and the suffix will solve the difficulty. In the examples given, it would not be possible to have a double bond between carbon atom 3 and carbon atom 5 and the suffix "-diene" shows that two double bonds are present. Use of $\Delta^{8,9}$ or $\Delta^{8,14}$ and the suffix "-ene" definitely limits the compound to one double bond between the carbon atoms indicated. Use of $\Delta^{8,14}$ and the suffix "-diene" would indicate double bonds between carbon atoms 8 and 9 and between carbon atoms 14 and 15.

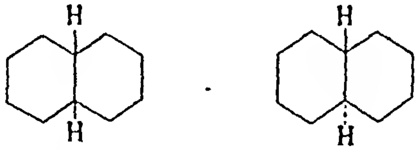
There is no general agreement about the superscripts used with Δ. The system explained here is used generally in this country. More complex situations than those illustrated may arise and necessitate some modification of this simple usage. The estrogens are a case in point, as will be shown later.

It is not essential to use Δ. The compounds in the examples given may

be named 5-androstene, 4-pregnene-3,20-dione, 3,5-androstadiene-17-one, and 5,16-pregnadiene. In "androstadiene" and "pregnadiene" an *a* has been added between the root and the suffix for euphony.

ISOMERISM INVOLVING CARBON ATOMS COMMON TO TWO RINGS

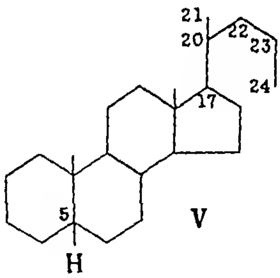
When saturated rings are fused in the manner shown in Fig. 2 (III), that is, with two carbon atoms common to both rings, two isomers are



III IV

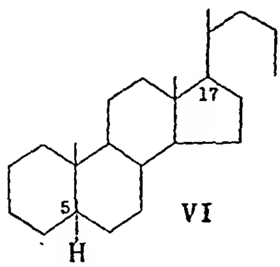
FIG. 2

possible depending on whether the hydrogen atoms attached at the junctures of the rings are on the same side (cis as in III) or on opposite sides (trans as in IV) of the plane of the rings. In the steroid ring system this type of isomerism may involve carbon atoms 5 and 10, 8 and 9, and 13 and 14. The relative arrangement of the hydrogen atom or methyl group on each of these atoms may determine two isomers for each carbon atom. Therefore the number of possible isomers is 2^6 or 64. Fortunately, this type



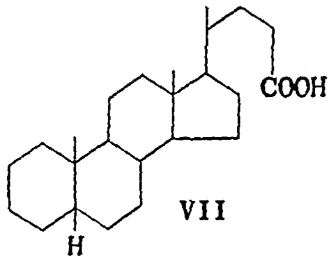
V

Cholane



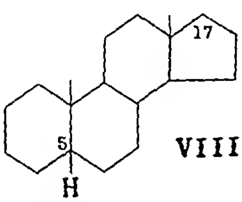
VI

Allocholane



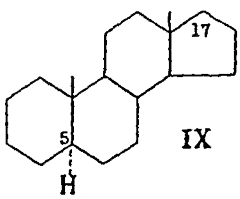
VII

Cholanic Acid



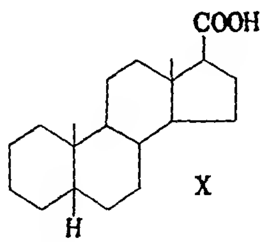
VIII

Etiocholane



IX

Etioallocholane (Androstane)



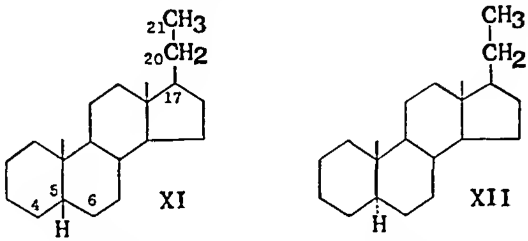
X

Etiocholanic Acid

FIG. 3

of isomerism is limited to C-5 in the commonly occurring steroids related to hormones.

In the hydrocarbon cholane (Fig. 3, V), which has the carbon skeleton of the bile acids, the methyl group attached to C-10 and the hydrogen atom at C-5 are considered to be on the same side of the ring system (cis relation). The hydrocarbon with the hydrogen atom at C-5 having the opposite (trans) arrangement with relation to the methyl group at C-10 is called allocholane (VI). In formula V, the bond holding the hydrogen atom at C-5 is written with a solid line to show that the hydrogen atom is on the same side of the ring system as the methyl group at C-10 (above the plane



XI Pregnane

XII Allopregnane

FIG. 4

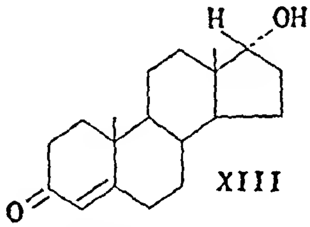
of the paper). In formula VI, the bond at C-5 is written with a broken line to show that the hydrogen atom is on the opposite side of the ring system from the methyl group at C-10. In the naturally occurring steroids, the methyl groups at C-10 and C-13 are considered always to have the same relation to the rest of the molecule, that is, above the plane of the molecule as written, and the bond is shown as a solid line.

If the chain of carbon atoms attached at C-17 in cholane and allocholane is removed, the corresponding hydrocarbons are etiocholane (VIII) and etioallocholane (IX). Etioallocholane is called more commonly "androstane." The prefix "etio-," as used in steroid chemistry, indicates that the compound is the simplest one that can be obtained by degradation of the parent substance and still retain the steroid ring system and the fundamental chemical characteristics of the parent substance. Thus, etiocholane is the steroid ring system with the two methyl groups whereas etiocholanic acid (X) has a carboxyl group at C-17. Degradation of etiocholanic acid by one carbon atom more would not give an analogue of cholanic acid (VII) but a neutral compound (etiocholane).

The hormones and related compounds may be named as derivatives of etiocholane or of androstane (etioallocholane). This system is used by Selye. It is often more convenient, however, to name some compounds as derivatives of pregnane (Fig. 4, XI) and allopregnane (XII), which are

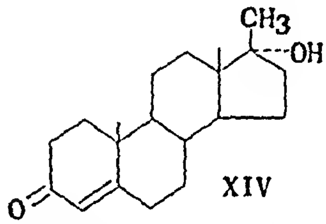
17-ethyl derivatives of etiocholane and etioallocholane (androstane), respectively.

It will be noted that in pregnane (or allopregnane) the numbers jump from 17, the last carbon atom in the ring system, to 20, the first carbon atom in the side chain and that C-17 and C-20 are joined together. The missing numbers, 19 and 18, are assigned to the two methyl groups attached at C-10 and C-13. These numbers have not been included since there is some disagreement as to which methyl group should receive which number. It might be pointed out, however, that it would seem to be logical to assign the number 18 to the methyl group at C-13 and 19 to the methyl



XIII

Testosterone



XIV

17-Methyltestosterone

FIG. 5

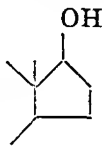
group at C-10 since the methyl group at C-10 is missing in the estrogens. If the methyl group at C-10 is numbered 18 and that at C-13 is numbered 19, then there would be a gap in the numbering system of the estrogens.

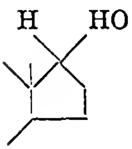
A double bond at 4-5, as in testosterone (Fig. 5, XIII) or progesterone (Fig. 8, XX), or at 5-6, as in dehydroisoandrosterone (Fig. 7, XVII), eliminates the possibility of isomerism at C-5 and the simpler name may be used with the ending "-ene"; examples, androstene, pregnene.

(α) AND (β) CONFIGURATIONS

Other important points at which isomerism may occur are at C-3, C-11, and C-20. At C-3 and C-11 the two valences of the carbon atom that are not attached to other carbon atoms are so arranged that one extends above and the other below the plane of the ring system. Substitution of one of the hydrogen atoms with OH, for example, offers two possibilities. The OH may be above or below the plane of the rings and thus two different compounds are possible. Since both angular methyl groups are considered to be on the same side of the ring system and, by convention, to be above the plane of the ring system as it is ordinarily written in the plane projection, it is customary to refer the configuration of all substituents to one or the other methyl group. Those substituents that are on the same side of the ring system as the methyl groups have the (β) configuration and those

on the opposite side the (α) configuration. In accord with the convention used to indicate the position of the hydrogen atom at C-5 the (α) configuration is ordinarily shown by a broken line for the bond connecting the substituent to the carbon atom in question while the (β) configuration is indicated by use of a solid line for the bond.

The use of a solid bond to indicate the (β) configuration is somewhat ambiguous, since a solid line is ordinarily used to indicate a bond without any implications as to configuration. In many cases the configuration is unknown. Therefore care must be used in the interpretation of formulas in which (α) and (β) positions are indicated by dotted and solid lines, respectively. A solid bond may mean only that the configuration is unknown. This difficulty could be avoided by showing both atoms or groups attached to the carbon in question. For example, 

may be ambiguous but 

could mean only that the configuration is not specified (and therefore presumally unknown) since the H and OH cannot both be in the (β) position.

In the name of a compound the configuration of a substituent is shown by writing α or β in parentheses after the number of the carbon atom involved. Example: Δ^5-androstene-3(β),17(α)-diol. "Trans" and "cis" are sometimes used interchangeably with (α) and (β), respectively, since substituents in the (α) position are on the opposite side of the molecule from the methyl groups and those in the (β) position are on the same side. However, this usage is not always consistent. For example, dehydroisoandrosterone has also received the name trans-dehydroandrosterone but the OH at C-3 (to which "trans" refers) has the (β) configuration. In this instance "trans" is not used with reference to the methyl group at C-10.

Epimers are isomers which differ only in the steric arrangement of the groups about one carbon atom. The prefix "epi-" is used to indicate such a relation. Androsterone and isoandrosterone are epimers and the latter could be called "epiandrosterone." In names of steroids the prefix "iso-" often is used interchangeably with "epi-" as in the case of isoandrosterone. The carbon atom involved is indicated in the usual way if more than one carbon atom may be involved. Thus, if the 12-hydroxyl group of desoxycholic acid is caused to assume the configuration opposite to the natural one, the resulting compound would be 12-epidesoxycholic acid or 12-iso-

desoxycholic acid. It should be added, however, that the prefix "iso-" may be used to indicate any type of isomerism in organic chemistry.

With reference to the steroids under consideration the prefix "allo-" (Greek allos, other) usually refers specifically to isomerism at C-5. This type of isomerism was illustrated by cholane (Fig. 3, V) and allocholane (VI) and their derivatives. Cholane, etiocholane (VIII) and pregnane (Fig. 4, XI) are considered to have the "normal" arrangement at C-5. Compounds with the opposite arrangement at C-5 are allo compounds such as etioallocholane (androstane).

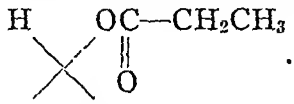
The terms "epi-," "iso-," and "allo-" have other connotations in organic chemistry and even with reference to steroids (for example, "allocholesterol" which has no hydrogen atom at C-5). However, they have the specific meanings indicated in connection with the compounds discussed in this paper.

With these principles in view we may proceed to consider specific applications.

ANDROGENS AND RELATED SUBSTANCES

Testosterone (Fig. 5, XIII) is the trivial name of the compound generally considered to be the androgenic hormone produced by the testis. A systematic name would be Δ^4-androsten-17(α)-ol-3-one or Δ^4-etiocholen-17(α)-ol-3-one. With a double bond at 4-5 androstene and etiocholene are identical. Given the name, one can write the structural formula by drawing the steroid ring system with the angular methyl groups, placing a ketone group at C-3, a double bond between carbon atoms 4 and 5 and a hydroxyl group attached to C-17 with a broken line. The group involving C-3 to C-5 is generally called an α,β-unsaturated ketone group and is characteristic of all of the most active steroid hormones with the exception of the estrogens. Change of this group in any detail reduces or abolishes the specific activity of the hormone in question.

Testosterone propionate usually is used for therapy. In this compound a molecule of propionic acid has been combined with the 17-OH to form

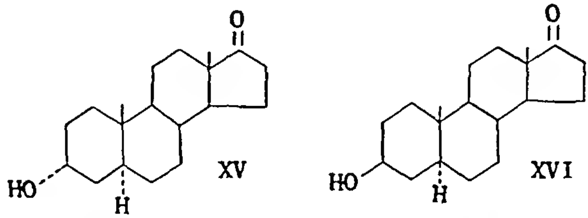
the ester group. 

. Since there is only one hydroxyl group to enter into ester formation it is not necessary to be more specific but if there were more than one hydroxyl group present it would be necessary to specify with a number which hydroxyl group is involved.

An isomer of testosterone is known which has the 17-OH in the (β) position. It is called cis-testosterone since a 17-OH in the (β) position has the cis relation to the methyl group at C-13, that is, these two groups are on the same side of the molecule. Another name for cis-testosterone is Δ^4-androsten-17(β)-ol-3-one.

The synthetic compound, methyltestosterone (XIV), has a methyl group at position 17 as well as a hydroxyl group and would properly be named "17-methyltestosterone" to indicate the position of the methyl group. A systematic name would be "17-methyl-Δ^4-androsten-17(α)-ol-3-one." Other androgens have been isolated from adrenal extracts: Δ^4-androstene-3,17-dione, 11(β)-hydroxyisoandrosterone (androstane-3(β), 11(β)-diol-17-one) and adrenosterone (Δ^4-androstene-3,11,17-trione). It is possible that these substances are artefacts produced during the process of isolation but there is no evidence on which to base a decision.

The known metabolites of testosterone are included in the group of

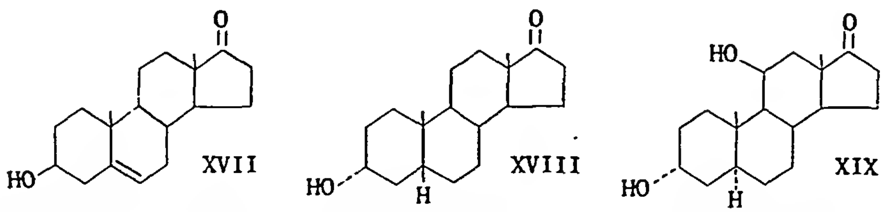


XV Androsterone

XVI Isoandrosterone

FIG. 6

compounds known as the "neutral" (to distinguish them from estrogenic phenols) 17-ketosteroids. These compounds are so named because of the presence of a ketone group at C-17. All of the common ones also have a hydroxyl group at C-3. The trivial name "androsterone" (Fig. 6, XV) has been given to androstan-3(α)-ol-17-one (or etioallocholan-3(α)-ol-17-one). Its isomer with the 3-hydroxyl group having the (β) configuration but with



XVII Dehydroisoandrosterone

XVIII Etiocholan-3(α)-ol-17-one

XIX 11(β)-Hydroxyandrosterone

FIG. 7

the rest of the molecule identical is isoandrosterone, or androstan-3(β)-ol-17-one (XVI). If a double bond is introduced in isoandrosterone by loss of a hydrogen atom from C-5 and one from C-6 (dehydrogenation) the resulting compound is dehydroisoandrosterone (more specifically 5-dehy-

droisoandrosterone) or Δ^5-androsten-3(β)-ol-17-one (Fig. 7, XVII). Dehydroisoandrosterone sometimes is named "trans-dehydroandrosterone" (*t*-dehydroandrosterone) but the former term is preferred. This substance and isoandrosterone comprise the major portion of the (β) fraction (so called because of the (β) configuration of the 3-hydroxyl group) of the 17-ketosteroids. The amount of the former compound is greatly increased in most patients who have adrenal cortical tumors and therefore a determination of the (β) fraction as well as the total amount of 17-ketosteroids may be helpful for differentiation between the presence of a tumor and cortical hyperplasia.

Another isomer of androsterone is etiocholan-3(α)-ol-17-one (XVIII). This compound differs from androsterone only in the configuration at C-5 The difference is sufficient to abolish all androgenic activity in etiocholan-3(α)-ol-17-one. The name of this compound also sometimes is written "3(α)-hydroxyetiocholan-17-one" and even more simply as "etiocholanolone." The latter name is indefinite and should be used only when there is no possibility of mistake as to the compound meant. The name "3(α)-hydroxyetiocholan-17-one" is an example of the use of a prefix to indicate one substituent and the use of a suffix to indicate another substituent.

Another 17-ketosteroid found in normal urine is 11(β)-hydroxyandrosterone or androstane-3(α),11(β)-diol-17-one (XIX). This substance is generally considered to be a metabolite of adrenocortical steroids, since it has the oxygen atom at C-11 which is characteristic of certain cortical hormones. Some of the other 17-ketosteroids, particularly dehydroisoandrosterone, and part of the androsterone and etiocholanolone, are derived from adrenal steroids but their relation to the known adrenal compounds is obscure.

Urine also may contain alcoholic steroids without a ketone group which are related to the 17-ketosteroids. Δ^5-Androstene-3(β),17(α)-diol, etiocholane-3(α),17(α)-diol, and Δ^5-androstene-3(β),16(β),17(α)-triol are such compounds.

PROGESTERONE AND RELATED COMPOUNDS

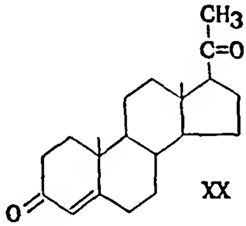
Progesterone (Fig. 8, XX) is Δ^4-pregnene-3,20-dione. According to the system used by Selye, (1) it may be named as a derivative of androstene: 17(β)-[1-ketoethyl]-Δ^4-androsten-3-one. It should be noted, however, that the 1-ketoethyl side chain is now known to be attached in the (β) position rather than in the (α) position as given by Selye.

The chief metabolite of progesterone is pregnane-3(α),20(α)-diol (XXI), which occurs in relatively large amounts in the urine of pregnancy. The name is often shortened to "pregnanediol." Unless otherwise indicated, this name may be interpreted to mean the 3(α),20(α)-diol. The other three

possible isomers are known and pregnane-3(β),20(α)-diol, which occurs in minor quantities, probably also is a metabolite of progesterone.

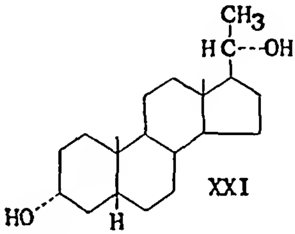
The designation '20(α)" does not have the same structural significance as, for example 3(α) or 17(α) since C-20 is in the side chain. However, the bond between C-20 and the OH is often shown as a broken line to indicate the (α) compound or as a solid line to indicate the (β) compound. In this case (α) and (β) serve to identify certain compounds but the precise relation of the substituent at C-20 to the rest of the molecule is uncertain.

The name "pregnanediol" often is spelled "pregnandiol," without the *e* of the "-ane" suffix, and is pronounced "preg-nan′-dee-ol." According to



XX

Progesterone



XXI

Pregnane-3(α),20(α)-diol

FIG. 8

the principles discussed at the beginning of this paper, this spelling and pronunciation obviously are not correct. "Prĕg′-nāne-dī′-ōl" indicates immediately the chemical nature of the compound whether written or spoken.

Pregnan-3(α)-ol-20-one also is a metabolite of progesterone. Recently it has been found to be an appreciable fraction of the sodium pregnanediol glucuronidate of pregnancy urine as ordinarily isolated (2).

Δ^5-Pregnen-3(β)-ol-20-one, usually named simply "pregnenolone," has been isolated from hog testes (3). It is reported to have a favorable effect on endurance and fatigue (4) and to be effective in maintaining testicular weight and spermatogenesis in hypophysectomized rats (5).

ADRENOCORTICAL HORMONES

All of the characteristic adrenocortical steroids that have been isolated are derivatives of pregnane. The chemical details of those known to have physiologic activity are summarized in Table 2. Progesterone and estrone have been isolated from adrenal extracts but they are not included in Table 2, since they are also ovarian products. Androgenic substances with the same number of carbon atoms as androsterone also have been isolated from adrenal extracts. They are considered with the other androgens. 17-Hydroxyprogesterone is included with the adrenal hormones because of its

close structural relationship, although its function is unknown beyond the fact that it has androgenic properties (6).

The first adrenocortical hormone to receive a trivial name was corticosterone and the other hormones have been named in systematic fashion as derivatives of corticosterone. Referring to Table 2, "11-desoxy-" means

TABLE 2. ADRENOCORTICAL HORMONES

Ordinary name	Chemical details		
	C=O	OH	Double bond
11-Desoxycorticosterone	3,20	21	4–5
Corticosterone	3,20	11, 21	4–5
11-Dehydrocorticosterone	3,11, 20	21	4–5
17-Hydroxycorticosterone	3,20	11, 17, 21	4–5
11-Dehydro-17-hydroxycorticosterone	3,11, 20	17, 21	4–5
17-Hydroxyprogesterone	3,20	17	4–5

that an oxygen atom of corticosterone (Fig. 9, XXII and XXIII) has been removed and replaced by hydrogen at C-11. "11-Dehydro-" means that two hydrogen atoms have been removed at C-11. Since corticosterone (XXII) has OH at C-11, removal of two hydrogen atoms converts the >CHOH group to a >C=O group (XXIV). The prefix "dehydro-" may mean also that a hydrogen atom has been removed from each of two adjacent carbon atoms with establishment of a double bond. This use was illustrated in the name "dehydroisoandrosterone" (Fig. 7, XVII). By reference to Table 2 and formulas XXII to XXIV (Fig. 9) the structure of the other hormones can be constructed readily.

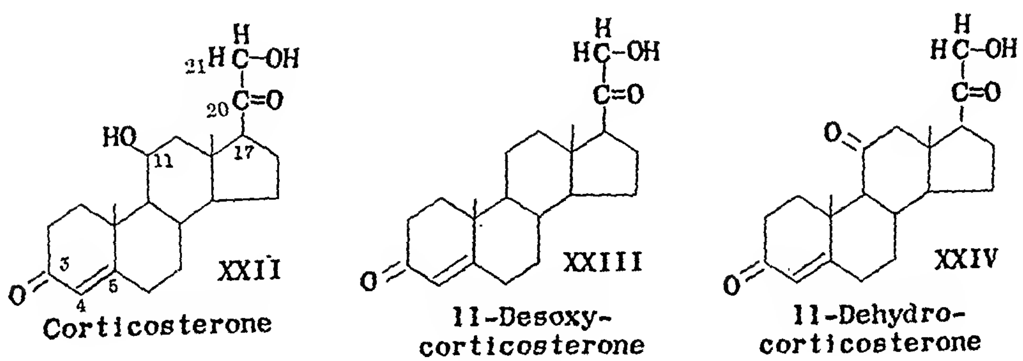
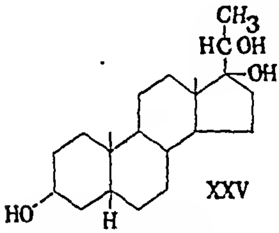


FIG. 9

11 Desoxycorticosterone (usually named simply "desoxycorticosterone") acetate is used widely for therapy in Addison's disease. In this compound a molecule of acetic acid has been combined with the OH group at C-21 to give an ester. This compound could properly be named "11-desoxycorticosterone 21-acetate."

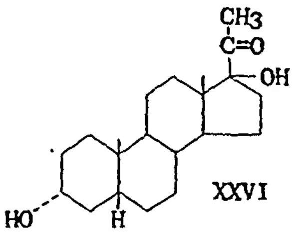
Many other compounds have been isolated from adrenal extracts but only those that are known to have physiologic activity are considered here.

In all of the adrenal steroids, including those that are not active hormones, if a hydroxyl group is present at C-11 it has the (β) configuration. Also, the side chain at C-17 has the (β) configuration. Therefore the 17-hydroxyl group in 17-hydroxycorticosterone, 11-dehydro-17-hydroxycorticosterone and 17-hydroxyprogesterone must have the (α) configuration. A more systematic name for 17-hydroxycorticosterone would be "Δ^4-pregnene-11(β),17(α),21-triol-3,20-dione," or, according to the system used by Selye, "17(β)-(1-keto-2-hydroxyethyl)-Δ^4-androsten-3-one-11(β),17(α)-diol." The latter name differs from that given by Selye in that the configurations assigned to the side chain and the hydroxyl group at C-17 are reversed in accordance with more recent evidence on the configurations at C-17. Note the order of "-one" and "-diol."



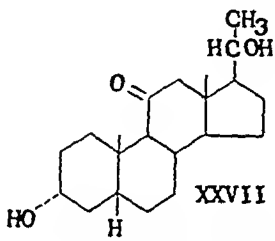
XXV

Pregnane-3(α), 17,20-triol



XXVI

Pregnane-3(α),17-diol-20-one



XXVII

Pregnane-3(α),20-diol-11-one

FIG. 10

The term "11-oxysteroids" has been applied recently to urinary material which has the ability to stimulate deposition of glycogen in fasting adrenalectomized rats or mice. This property is characteristic of the adrenal hormones which have an oxygen atom at C-11. Although it may be logical to assume that the urinary material which has this type of physiologic activity has an oxygen atom at C-11 it is undesirable to assign to it a name which includes a specific structural feature when the exact nature of the material is still unknown. The terms "corticoid," "corticosterone-like" and "11-oxycorticosteroid-like" have also been applied to the urinary material. These terms imply only that the urinary material has properties like those of some of the adrenocortical hormones.

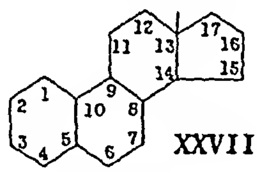
Probable metabolites of adrenal steroids are pregnane-3(α),17,20-triol (Fig. 10, XXV) and pregnane-3(α),17-diol-20-one (XXVI). Pregnane-3(α),20-diol-11-one (XXVII) (11-ketopregnanediol) is a metabolite of 11-dehydrocorticosterone and pregnane-3(α),20(α)-diol (Fig. 8, XXI) is a metabolite of 11-desoxycorticosterone as well as of progesterone. In XXV and XXVII (Fig. 10) the 20-hydroxyl group probably has the same configuration as in the ordinary pregnane-3(α),20(α)-diol but this has not been established definitely. Also, the OH at C-17 most probably has the (α) configuration but the configuration has not been proved definitely.

ARTEFACTS

Usually it is necessary to boil urine with mineral acids in order to hydrolyze the conjugates of steroids with sulfuric and glucuronic acids. This procedure is known to produce some unsaturated compounds which result from loss of a molecule of water. Such a compound is Δ^2- or Δ^3-androsten-17-one, which is derived from a conjugate of androsterone. The position of the double bond is uncertain. Others are $\Delta^{3,5}$-androstadien-17-one which is derived from dehydroisoandrosterone, and $\Delta^{9,11}$-androsten-3(α)-ol-17-one, which is derived from androstane-3(α),11(β)-diol-17-one.

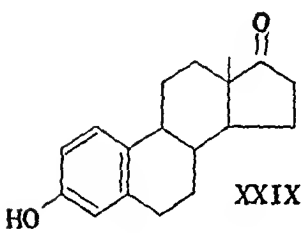
ESTROGENS

In the estrogens, ring A of the steroid nucleus has become an aromatic ring; that is, essentially a benzene ring with three double bonds. In order



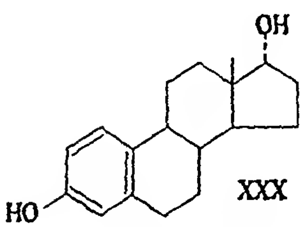
XXVIII

Estrane



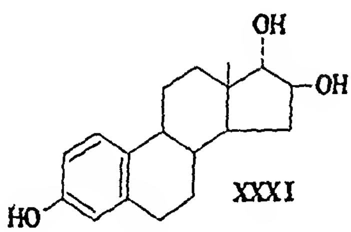
XXIX

Estrone



XXX

(α)-Estradiol



XXXI

Estriol

FIG. 11

for this ring to accommodate three double bonds the methyl group at C-10 has been lost. The aromatic character of this ring gives the hydroxyl group at C-3 the properties of a phenolic hydroxyl group. It has weakly acidic properties.

The saturated hydrocarbon which may be regarded as the parent substance of the estrogens is named "estrane" (Fig. 11, XXVIII). It is almost identical with etiocholane but lacks the methyl group at C-10. The numbering system is the same as for etiocholane. Inspection of the formula of estrone (XXIX) shows that it has three double bonds, a hydroxyl group and a ketone group. Its systematic name, therefore, is "$\Delta^{1,3,5:10}$-estratrien-3-ol-17-one." α-Estradiol (XXX) is $\Delta^{1,3,5:10}$-estratriene-3,17(α)-diol and estriol (XXXI) is $\Delta^{1,3,5:10}$-estratriene-3,16(β),17(α)-triol. Equilin and equilenin, respectively, are $\Delta^{1,3,5:10,7}$-estratetraen-3-ol-17-one and $\Delta^{1,3,5:10,6,8:9}$-estrapentaen-3-ol-17-one. The superscripts 5:10 and 8:9 indicate the ends of the double bonds in these positions, since the use of 5 or of 8 alone would be ambiguous.

A more extensive discussion of steroid nomenclature will be found in the monograph by Fieser (7). This subject is also discussed in the books by Gilman (8) and Sobotka (9). Examples of trivial and systematic names of hormones and related steroids will be found in Selye's Encyclopedia (1).

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* This name would now be "17(α)-Hydroxyprogesterone."

Fred Conrad Koch
1876–1948

Obituary

★

FRED CONRAD KOCH

1876–1948

FRED CONRAD KOCH died at his home in Chicago on January 26, 1948. Not long before he had been ill with pneumonia but convalescence seemed to be progressing. Death, presumably from a heart attack, was unexpected. Those who mourn him may take some comfort from the circumstance that he was spared the long months of feebleness, disintegration of abilities, pain and anticipation of death that are so common. The image of full and friendly vigor left at our last meetings remains unimpaired.

Professor Koch's entire career developed within his native state. He was born in 1876 in Chicago. He studied at the University of Illinois, obtaining his bachelor's degree in 1899. After two further years there, teaching and working in organic chemistry, he went to Armour and Company as a research chemist. In 1909 his work took a new turn. The formal study of biochemistry was begun on a fellowship at the University of Chicago under A. P. Mathews. He received the Ph.D. degree in 1912. From then on until his retirement in 1941, he was affiliated with this department of biochemistry, serving as acting chairman from 1919 to 1926 and as chairman thereafter. His academic life culminated in 1941 with his appointment to the Frank P. Hixon Distinguished Service Professorship. When his university duties were over he resumed his old association with Armour and Company, serving as director of biochemical research. His career matured slowly but suffered no decline with advancing years. Well merited recognition came late after long arduous effort. He knew no true retirement.

His diversified contributions to endocrine research are well known to all. He will be best remembered, however, by his influence on the development of our understanding of testicular function. In 1926 he guided the work of a graduate student, L. C. McGee, in demonstrating the power of bull-testis extracts to induce growth of the capon's comb. With T. F. Gallagher and others this response was adapted to the quantitative measurement of the androgenic potency of tissue extracts, body fluids and pure steroids. With several others, the details of biological reactions to male hormone and hormone-like substances were described. Methods of extraction of active materials from urine were elaborated and an initial account given of the occurrence of androgens and estrogens in the urine of man. The discovery of androgenic activity in the urine of women was startling in its day and has set a perennial physiological problem. In his later years he was much concerned with pituitary functions and at Armour and Company accomplished the production of adrenotropins of high quality and in sufficient amounts for biological and clinical experiment. He gave attention to many other biochemical matters. With his wife and constant collaborator, Elizabeth Miller Koch, continuing studies on vitamin D were pursued.

Professor Koch was a devoted member of the Association for the Study of Internal Secretions, serving as its president in 1937. He received the coveted Squibb Award in 1942. Many will gratefully recall his tireless attention to the multitude of papers at our sessions, without regard to the greater or lesser fame of the speakers, and his unending courtesy to all in discussions after hours.

Claude Bernard once said, "Art is myself, science ourselves." This Professor Koch understood by temperament and instinct rather than from bitter lessons. As a great teacher he felt his highest honors in the success of his students. Their work now ramifies through many subjects, and much of it deals with endocrinology. From such seeds the harvest is incalculable. Here lies abundant fatherhood. The general sense of loss is tempered now by gratitude for what has been done.

ANNOUNCEMENT OF THE 1948 MEETING OF THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS

The Thirtieth Annual Meeting of the Association for the Study of Internal Secretions will be held in the Palmer House, Chicago, Illinois, June 18 and 19, 1948.

The scientific sessions will be held in the Red Lacquer Room and registration will be on the fourth floor just outside the Red Lacquer Room. The Annual Dinner will be held in the same room on Friday, June 18th at 7 P. M. and will be preceded by a cocktail party, the location of which will be announced later.

All members of the Association who plan to attend the Thirtieth Meeting are urged to make their reservations at once with the Palmer House, stating the time of arrival and how long they plan to remain in Chicago.

The program of the meeting will appear in the April or May issue of the Journal, and the abstracts of papers presented at the annual meeting will be published in the July issue.

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VOLUME 8 MARCH, 1948 NUMBER 3

STUDIES IN SERUM ELECTROLYTES. XVI. CHANGES IN THE SERUM AND BODY FLUIDS IN ANOREXIA NERVOSA

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AND EDWARD ROSE, M.D.

From the William Pepper Laboratory of Clinical Medicine, the Endocrine Section of the Medical Clinic, Hospital of the University of Pennsylvania, and Temple University Medical School, Philadelphia, Pa.

THE purpose of this paper is to report studies of changes in composition and distribution of the body fluids and serum electrolytes; assays of urinary hormones; and observations on chloride metabolism in a patient suffering from anorexia nervosa, whom we have followed for a period of three years. Wherever possible, our data are compared to those recently reported (1) pertaining to a severely emaciated individual who had undergone a voluntary fast for religious reasons for a period of 45 days. Such comparison would seem appropriate since the data might be regarded as pertaining to chronic and acute undernutrition, respectively.

The syndrome of anorexia nervosa has been so extensively described that no attempt will be made to review the literature or to repeat the clinical description of the disorder.

CASE REPORT

The patient, F. J., is a white, married woman, 29 years of age. At the age of 17 she weighed 112 pounds but became obsessed with the idea that she was overweight. She then began to reduce her caloric intake. During the next nine years her weight gradually fell to a minimum level of 56 pounds. She insisted that throughout this period her

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appetite remained normal and that she enjoyed her meals. For several years she had noted increasing constipation with much indigestion, characterized by flatulence and vague, generalized abdominal discomfort. During the past few years she frequently induced vomiting after meals. Although she complained of increasing weakness and fatigability, this was belied by her sprightly behavior during her hospital admissions.

The patient's menses began at the age of 17 but were very scanty and after a few months they ceased entirely for about two years. Scanty, irregular menstruation then recurred for about two years, but amenorrhea has been complete for the past six years. The patient married at the age of 21 but never became pregnant. Her libido was always minimal and for the past several years she has been completely frigid. Dyspareunia has been increasingly severe.

FIGURE 1

The patient's personality has been definitely abnormal. Although she talked at length about her complaints, she was vague regarding dates and time relationships. She quarreled with many of her physicians and has been bitterly critical of the various hospitals where she had stayed. During each of her several admissions to the medical wards of the Hospital of the University of Pennsylvania she quarreled with the nursing and dietetic staffs and with her fellow patients. She enjoyed the attention of students and physicians and was always eager to be presented at clinical lectures or to be the subject of laboratory tests. She had baffled the best efforts of several psychiatrists to discover the real basis of her behavior. From time to time she exhibited anxiety regarding the possible existence of organic diseases such as brain tumor, cholecystitis, or intestinal obstruction.

On physical examination the patient's appearance was one of striking emaciation without commensurate muscular weakness (Figure 1). She was 61 inches in height and weighed 56 pounds. Cranial, axillary, and pubic hair were normal in amount. The hair was somewhat dry. Hypertrichosis was present on the face, forearms, and back. The skin was dry and wrinkled with some scaling. Her blood pressure measurements averaged 110/80 mm. of Hg. The patient's pulse rates were normal but her oral temperatures were subnormal throughout most of the period of our observations, recordings between 96° and 97° F. being obtained almost daily. The mucous membranes of the mouth and pharynx were pale and smooth. Dental caries was widespread and she had only four teeth remaining in her lower jaw. No subcutaneous adipose tissue was apparent and the breasts were atrophic. The uterus and ovaries showed marked atrophy. There was unusual prominence and distention of the peripheral veins.

The blood examinations obtained on her several admissions since 1944 have shown a slight anemia. The hemoglobin concentrations ranged from 11.8 to 14 Gm. per 100 ml. The erythrocyte counts varied from 3.4 to 4.0 million per cu. mm., and the leucocytes

from 5000 to 8500 per cu. mm. Routine urinalyses were normal. Serologic tests for syphilis gave negative reactions. The basal metabolic rates were slightly decreased without any corresponding increase in the concentration of serum cholesterol. For example, on one occasion when the basal metabolic rate was minus 19 per cent (the lowest measurement obtained), the serum cholesterol was 194 mg. per 100 ml.

X-ray examinations of the skull, pituitary fossa, sinuses, chest, gallbladder, and entire gastro-intestinal tract showed nothing abnormal. Water excretion tests for adrenal cortical function by the method of Robinson, Power and Kepler (2) were normal.

Ballistocardiographic studies by Dr. Isaac Starr showed an excessive circulation rating of plus 43 per cent estimated in terms of her actual weight but only plus 9 per cent when estimated in terms of her ideal weight.

It should be mentioned that the patient did not exhibit clinical evidence of avitaminosis at any time.

RESULTS

Our methods of analyses are given in a previous paper (1). The distribution of the serum components and comparisons with the fasted individual and the normal range of values are given in Table 1. It will be seen that in both the patient with anorexia nervosa and the fasted individual the con-

TABLE 1. SERUM ANALYSES

	F.J. (Anorexia Nervosa)	B.D. (Prolonged Fasting—45 days)	Normal
Cl	58	74.0	99–104 mEq./L.
CO_2	138	100	55–60 vol. per cent
Total Base	129	141	143–148 mEq./L.
K	3.0	—	3–4 mEq./L.
Ca	10.6	12.2	9–11 mg./100 ml.
Mg	2.8	2.7	1.9–2.0 mEq./L.
P (Inorg.)	3.7	3.4	3–4 mg./100 ml.
Protein	6.3	6.2	6–7 Gm./100 ml.
Albumin	4.6	5.0	3.3–4.3 Gm./100 ml.
Globulin	1.7	1.2	2.2–2.8 Gm./100 ml.
Cholesterol	245	198	170–190 mg./100 ml.
Ester. Chol.	200	184	90–114 mg./100 ml.
Urea Nitrogen	9	24	9–17 mg./100 ml.
Uric Acid	5.3	9.0	3–4 mg./100 ml.
Creatinine	1.6	1.4	1–1.2 mg./100 ml.
Glucose	62	63	80–110 mg./100 ml.

centrations of serum chloride were remarkably decreased. In our patient with anorexia nervosa, values of serum chloride between 50 and 60 mEq. per liter have practically always been obtained; in the fasted subject the concentration of serum chloride on the 45th day of fasting was 74 mEq.

per liter. Although our patient had vomited after meals on a number of occasions and especially when chlorides were administered intravenously, nevertheless, it would seem to us that the decreased concentration of serum chloride cannot be explained entirely on this basis. Moreover, a similar decrease in chloride was observed in the fasted individual who had not vomited during his fasting period.

It will be seen that the decreased concentration of serum chloride was compensated by an increase in the concentration of bicarbonate. Although this inverse relationship is unexplained, it seems reasonable to relate it to diminished intake of chloride with substitution of bicarbonate derived from endogenous sources. The decreased concentration of total base was due to a decrease in the concentration of serum sodium. Similar reduction in serum sodium concentration has been reported in Simmonds' disease

TABLE 2. ELECTROPHORETIC ANALYSIS OF SERUM, (F.J.), ANOREXIA NERVOSA

Total Protein	7.09 Gm. per 100 ml.
Albumin	4.50 Gm. per 100 ml.
Total Globulin	2.59 Gm. per 100 ml.
Alpha Globulin	0.49 Gm. per 100 ml.
Beta Globulin	0.92 Gm. per 100 ml.
Gamma Globulin	1.18 Gm. per 100 ml.

(3). It should be noted that the diminutions in the concentrations of serum chloride both in the patient with anorexia nervosa and in the fasted individual were much greater than the diminutions in serum total base. From these studies it may be inferred that the factors regulating the serum electrolytes are more concerned with the maintenance of a normal distribution of cations than of anions.

The increase in the magnesium concentration in the serum of both individuals has been of particular interest. Hypermagnesemia is reported as a characteristic finding in animals during hibernation (4) and has recently been reported (5) in an infant with a hypothalamic lesion having subnormal temperature and a depressed metabolic state.

The concentrations of the total serum protein were normal both in the patient with anorexia nervosa and in the fasted subject. It will be observed however, that upon salting out the globulins, the albumin fractions in both individuals were greater than normal, thus yielding exceptionally high albumin-globulin ratios.

In Table 2 is given the electrophoretic analysis of a sample of serum.

It should be noted that in this sample the concentrations of the four components were essentially within the normal range of values.

The total cholesterol in the serum reported in Table 1 was 245 mg. per 100 ml. and the esters were over 80 per cent of the total. Since both serum albumin and cholesterol esters are probably synthesized by the liver, the increased levels of these components suggest that there was an unusual degree of hepatic activity during which these components were liberated. That this seems reasonable is suggested by the studies of Addis, Poo and Lew (6) who found that the livers of rats may lose as much as 40 per cent of their original protein after a seven day fast.

Of the nonprotein nitrogen components the uric acid and creatinine

TABLE 3. FLUID DISTRIBUTION

	F.J. Anorexia Nervosa	B.D. Prolonged Fasting	Normal
Serum Volume	ml.	ml.	ml.
ml./Kg. present weight	68.0	57.7	43–48
ml./Kg. initial weight	38.9	37.8	
Total serum volume	1856	2539	
Extracellular Water	Per cent	Per cent	Per cent
(present weight)	17.6	33.4	20–25
(initial weight)	10.1	23.6	

concentrations were increased in both individuals. The concentration of urea nitrogen was increased in the fasted subject but not in the patient with anorexia nervosa. Increase in the concentrations of the nonprotein nitrogen components has been reported by a number of investigators during fasting (7, 8, 9) and would appear to be due to the increased catabolism of protein. The normal concentrations of urea nitrogen observed in anorexia nervosa may be due to minimal catabolism of protein. The concentrations of serum glucose during fasting were decreased below the normal range of values in both individuals.

In Table 3 are given the values for the serum volume and the volume of extracellular fluid (thiocyanate space). The values for serum volume in our patient with anorexia nervosa as well as in the fasted subject were greatly increased when calculated in relation to actual body weight. However, when calculated in relation to the initial or ideal weight, the values for serum volume were decreased below the normal range. The prominence and distention of the superficial veins, as well as the ballistocardiographic studies previously mentioned, suggest that the volume of the circulatory

bed had not been greatly altered. In this connection it might be mentioned that during starvation the heart and brain lose percentilely less weight than other organs (10).

The difference in the volume of extracellular fluid between the patient suffering from anorexia nervosa and the fasted subject was striking. In the fasted subject the extracellular fluid volume was elevated in relation to his fasting weight but when calculated in relation to his prefasting weight it was within the normal range of values. On the other hand, the patient suffering from anorexia nervosa revealed a striking percentile decrease in the extracellular fluid volume in relation to her actual weight and obviously in relation to her ideal weight. It seems possible, therefore, that this dissimilarity may be due to the differences in the lengths of time during which these conditions lasted.

TABLE 4. ASSAYS OF URINARY HORMONES
(24-hour excretion)

	F.J. Anorexia Nervosa	Normal
Gonadotropins	none	8– 30 m.u.
Estrogens	<6 m.u.	22–100 m.u.
Neutral 17-Ketosteroids	2.3 mg.	9– 15 mg.

Assays of the urinary hormones in the patient with anorexia nervosa are given in Table 4. As might have been anticipated the excretion of gonadotropins was minimal;—in fact none was detected in 24-hour samples of urine. The excretion of estrogens and neutral 17-ketosteroids was very low.

In Table 5 are given the results of the insulin, galactose and glucose tolerance tests in our patient. Fraser and his colleagues (11) have suggested the use of the insulin sensitivity test in the differential diagnosis between pituitary cachexia (Simmonds' disease) and anorexia nervosa. According to these investigators, in normal individuals and in patients with anorexia nervosa the concentrations of fasting blood sugar are within the normal range of values; one-half hour after giving intravenously 0.1 unit of insulin per kilogram of body weight, the concentration of blood sugar is decreased, returning to the normal fasting level within two hours. On the other hand in pituitary cachexia, the concentration of blood sugar is decreased at the end of one-half hour and remains decreased until after two hours. On three occasions in which this test was tried in our patient, the concentrations of blood sugar were decreased below the normal range at the one-half hour

period and remained so for two hours. In spite of these positive insulin tolerance tests we believe that our patient was probably suffering from anorexia nervosa rather than from an organic lesion of the anterior pituitary gland (Simmonds' disease). However, the differentiation between anorexia nervosa and Simmonds' disease is an extraordinarily complex one. Whether minor destructive lesions occur in the pituitary gland in anorexia nervosa and indeed whether the two diseases are independent of each other is speculative at the present time. In Table 5 it will be observed that in one

TABLE 5. TOLERANCE TESTS, (F.J.), ANOREXIA NERVOSA

	Blood Sugar mg./100 ml.		
Insulin Tolerance Test	7/13	7/19	11/27
Fasting	58	69	66
5 minutes	66	—	—
30 minutes	44	40	16*
1 hour	—	12*	32
2 hours	—	46	57
Glucose Tolerance Test (2-dose)			
Fasting	62	83	
30 minutes	128	95	
1 hour	119	92	
Galactose Tolerance Test		Blood Galactose (mg. per 100 ml.)	
5 minutes	11		
30 minutes	15		
1 hour	4		

* Symptoms of shock.

of the glucose tolerance tests the concentration of blood sugar did not rise to the usual level observed in normal individuals. In this connection it should be noted that although the concentrations of the fasting blood sugar have been frequently decreased in our patient, at no time did she manifest symptoms of spontaneous hypoglycemia. In anorexia nervosa, in contrast to Simmonds' disease, spontaneous hypoglycemia has not been reported.

The galactose tolerance test according to the method of Althausen (12) was essentially normal.

CHLORIDE BALANCE

Of particular interest was the finding that our patient continued to excrete chloride in the urine even when the concentration of serum chloride

TABLE 6. CHLORIDE BALANCE AND SERUM ANALYSES

Dates	Intake Gm. NaCl			Output Gm. NaCl			*Balance Gm. NaCl	Cl mEq./L	CO_2 Vol. per 100 ml.	Total Protein Gm. per 100 ml.	Total Base mEq./L	Cl^- mEq./l.	HCO_3^- nM/L	Pr^- nM/L	Measured Anions mEq./L	Remarks
	Diet	Intra-venous	Total	Urine	Vomitus	Total										
1/27	3.25 (55.6)*	10.81 (184.9)	14.06 (240.6)	0.19 (3.3)	7.63 (130.5)	7.82 (133.8)	+6.24 (+106.8)	64.0	119	6.4	123.8	64.0	51.8	13.0	128.8	
1/28	3.10 (53.0)	13.74 (235.1)	16.84 (288.1)	0.66 (11.3)	8.59 (147.0)	9.25 (158.3)	+7.59 (+129.8)	94.0	85	4.6	136.0	94.0	26.7	9.3	130.0	
1/29	3.80 (65.0)	12.91 (220.9)	16.71 (285.9)	1.00 (17.1)	10.86 (185.8)	11.86 (202.9)	+4.85 (+83.0)	92.1	82	4.4	136.0	92.1	25.3	8.9	126.3	Discontinued on account of edema
1/30	4.34 (74.3)	11.23 (192.1)	15.57 (266.4)	0.86 (14.7)	16.13 (276.0)	16.99 (290.7)	−1.42 (−24.3)									
1/31	4.60 (78.7)	4.35 (74.4)	8.95 (153.1)	0.87 (14.9)	11.63 (199.0)	12.50 (213.9)	−3.55 (−60.8)									

* Values in parentheses represent chloride expressed as mEq.

was between 50 and 60 mEq. per liter. The amounts of chloride excreted were small, to be sure; however, they were readily measurable and ranged from 0.25 to 0.5 grams of NaCl (4.3 to 8.6 mEq. chloride) per day. Normally from 6 to 12 grams of chloride as NaCl (100 to 200 mEq. chloride) are excreted in the urine in 24 hours. Chloride usually disappears from the urine, except for traces, when the serum chloride falls to levels below 80 mEq. per liter. Although the so-called renal threshold for chloride is probably not as definite a level as was once believed, nevertheless, there is undoubtedly a normal tendency for the body to conserve chloride and to prevent its excretion in the urine when the chloride concentration in the serum is decreased below the normal range of 98 to 104 mEq. per liter. It would appear that the threshold for excreting chloride in our patient with anorexia nervosa has been remarkably reduced. Although the water excretion test for adrenal function was never positive, the urinary excretion of neutral 17-ketosteroids was low. This suggests some functional insufficiency of her adrenal cortex. Such insufficiency may possibly have reduced the ability of the renal tubules to reabsorb chloride and thus may have contributed to the reduced levels of serum total base and chloride.

In Table 6 are given data pertaining to the measurements of chloride balance and concentration of serum components undertaken at a time when the patient was given NaCl intravenously. Soon after commencing saline infusions, the patient began to vomit. It will be seen that during the five day period of this therapy, the patient showed a positive chloride balance from 4.85 to 7.59 grams of NaCl (83 to 130 mEq. chloride) per day during the first three days. Since the patient was constipated, the amount of Cl excreted in the stool, which is presumably less than 0.5 grams NaCl (8.6 mEq. chloride) per day, was not included. At the time of these studies the concentration of serum chloride increased from 64.0 to 94.0 mEq. per liter and the total serum CO_2 decreased from 119 to 85 volumes per cent. The concentration of total serum protein also decreased to 4.6 grams per 100 ml. During the next two days vomiting became increasingly severe and the vomitus contained increasing amounts of chloride. Moreover, the patient became strikingly edematous. The intravenous administration of NaCl was therefore discontinued. Within two weeks thereafter, the patient lost her edema, and her serum electrolytes reverted to their previous pattern.

COMMENT

The pattern of our patient's personality conformed closely to that seen typically in anorexia nervosa. Moreover, we were unable to discover or to eradicate the basic psychic abnormality which was almost certainly the cause of her initial compulsion to restrict her diet at a time when she weighed

only 112 pounds. The gradual replacement of a reduced caloric intake by prolonged periods of fairly normal diet accompanied by postprandial vomiting emphasizes the ineptness of the term "anorexia nervosa." Anorexia in the true sense of the term is by no means always seen, even when undernutrition results from reduced food intake rather than from habitual vomiting. The dietary restriction often results more from the exercise of will than from actual distaste for food. Likewise, the term "nervosa" is meaningless. The substitution of a more accurately descriptive term, such as "chronic psychogenic undernutrition," appears desirable. However, the term "anorexia nervosa," like many other vague and inaccurate descriptive names in medicine, has become so deeply rooted by custom that its elimination seems well nigh impossible.

Our patient showed the usual disparity between the degree of cachexia and the extent of actual muscular asthenia. Controversy has long existed concerning the relative importance of psychic factors versus physiologic derangement of certain organ systems, especially the endocrine, in some chronically undernourished patients. This difficulty has resulted in considerable confusion of terminology in the literature, particularly in regard to the diagnosis of pituitary cachexia. Many cases are described as examples of anorexia nervosa. The evidence in our case points toward the development of physiologic depression of the endocrine system probaby as a result of chronic inanition which in turn was due to primary psychic or emotional factors. In this sense, then, the existence of a true functional hypopituitarism in the later stages of the disorder must be admitted, and such a functional hypopituitarism probably contributes in large part to the state of depression found in the other endocrine target-organs (thyroid, adrenals, gonads). Nevertheless, there remain in most cases both objective and subjective distinctions which usually make it possible to differentiate between the functional and the truly organic types of anterior pituitary deficiency. It, therefore, appears desirable to reserve the term "pituitary cachexia" (Simmonds' disease, panhypopituitarism, cachexia hypophyseopriva) for those cases in which organic destructive lesions of the anterior pituitary (including atrophy with fibrosis) can be demonstrated or reasonably inferred.

SUMMARY

Studies of the changes in composition and distribution of the body fluids and serum electrolytes have been made in a patient with anorexia nervosa. These have been compared with similar studies previously reported in a severely emaciated individual who had undergone a voluntary fast for a period of 45 days.

The concentrations of serum chloride were remarkably decreased in both

individuals. The decreased concentrations of chloride were compensated by increases in the bicarbonate. Although the concentrations of serum total base were decreased in both subjects, the decreases of total base were much less than those of chloride. It appears that the factors regulating the serum electrolytes are more concerned with maintenance of normal distribution of cations than of anions.

The concentrations of serum magnesium were increased in both individuals.

The concentration of serum albumin, cholesterol esters, uric acid and creatinine were increased in both subjects.

The serum volume in each subject was increased when calculated in relation to actual weight, but was decreased when estimated in relation to the normal weight.

The volume of extracellular fluid was decreased in the patient with anorexia nervosa. In the fasted subject the volume of extracellular fluid was increased in relation to his actual weight, but was within the normal range when calculated in relation to his prefasting weight.

The cardiac output (ballistocardiographic method) was increased in each subject when estimated in relation to actual weight, but was normal when estimated in relation to ideal weight.

The patient with anorexia nervosa continued to excrete chloride in her urine even when the concentration of serum chloride was below 60 mEq. per liter.

Measurements of chloride balance made during a period of intravenous administration of NaCl in the patient with anorexia nervosa showed a positive chloride balance for the first three days; during this period the concentration of serum chloride increased from 64.0 to 94.0 mEq. per liter.

The patient with anorexia nervosa showed increased sensitivity to insulin, lowered basal metabolic rates and decreased urinary excretion of estrogens, gonadotropins and neutral 17-ketosteroids. These findings suggest functional insufficiency of the anterior pituitary, gonads, and perhaps of the thyroid and adrenal cortex. The relationship between the syndrome of anorexia nervosa and functional insufficiency of the endocrine system has been briefly discussed.

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A DIURNAL RHYTHM IN THE EXCRETION OF NEUTRAL REDUCING LIPIDS BY MAN AND ITS RELATION TO THE 17-KETOSTEROID RHYTHM*

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THE observation that there is a diurnal rhythm in urinary ketosteroid excretion (1, 2, 3) and that this may be related to stress-stimulated adrenocortical secretion (2) led us to examine other possible indices of adrenocortical secretion. The development of a method for measuring neutral reducing lipid in urine by Heard and Sobel (4) and the indications that this material represents in large part corticosteroid possessing reducing activity by virtue of its α-ketol sidechain (4, 5, 6), offered an opportunity to assess the diurnal changes in excretion of adrenocortical substances, which are entirely different from those of the 17-ketosteroids.

MATERIAL AND METHODS

Twenty-four normal healthy men ranging in age from 24 to 72 years (see Table 1) were the subjects of this investigation. Urine was collected over a 24-hour period in three lots covering approximately the periods 11 p.m. to 7 a.m. (sleep), 7 a.m. to 11 a.m. (morning) and 11 a.m. to 11 p.m. (day).

From an aliquot of each collection, fractionation for the neutral ketones and 17-ketosteroid determination was made by methods previously described (7). A second aliquot was employed for determining neutral reducing lipid by the Heard-Sobel method (4). In our experience the urine of normal men ordinarily contains sufficient 17-ketosteroid and neutral reducing lipid to make the equivalent of a half-hour collection sufficient for accurate colorimetric determination. Creatinine determinations were made routinely on each specimen (7).

RESULTS

In Table 1 we present for each subject the urinary outputs of neutral reducing lipid and 17-ketosteroid respectively, on the basis of mg. per

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twenty-four hours and mg. per Gm. of creatinine. An inspection of the table indicates that for both types of steroid and on either measurement basis, the great majority of subjects have minimal outputs during the sleep period. Actually the 17-ketosteroid measurements on a 24-hour basis show only two men in whom the sleep value is not less than either of the waking values; on a gram-creatinine basis there are three such men. Similarly the sleep "cortin" output is highest in only four subjects on the 24-hour basis and in only two on a gram-creatinine basis.

TABLE 1. NEUTRAL REDUCING LIPID* ("CORTIN") AND 17-KETOSTEROID URINARY OUTPUTS OF NORMAL, HEALTHY MEN

Subject No.	Age Yrs.	Mg. per 24 Hours						Mg. per Gm. of Creatinine					
		"Cortin"			17-Ketosteroids			"Cortin"			17-Ketosteroids		
		Sleep	Morning	Day	Sleep	Morning	Day	Sleep	Morning	Day	Sleep	Morning	Day
1	24	1.06	2.58	1.74	6.24	18.72	9.36	0.56	1.26	0.72	3.27	9.15	3.88
2	24	2.42	2.10	2.13	4.32	12.24	16.56	1.01	0.69	1.02	1.80	4.01	7.92
3	24	1.39	2.91	1.71	14.16	29.76	16.32	1.00	1.29	1.14	10.20	13.20	10.90
4	26	0.71	0.77	1.46	11.28	6.72	14.16	0.38	0.39	0.80	5.96	3.44	7.74
5	27	0.62	2.32	1.51	9.60	12.72	7.92	0.57	1.69	1.18	8.90	9.30	6.18
6	27	1.56	1.83	3.23	14.16	16.32	15.84	1.00	1.33	1.63	9.08	11.85	8.00
7	27	2.03	2.34	1.02	12.96	19.68	19.20	1.48	1.18	0.55	9.45	9.93	10.40
8	28	0.58	1.71	1.39	8.88	4.80	10.56	0.38	1.20	0.70	5.75	3.36	5.28
9	29	0.98	2.42	1.49	11.28	20.16	8.88	0.54	1.21	1.05	6.27	10.08	6.25
10	30	1.17	2.40	1.75	13.20	24.24	21.36	0.54	1.35	0.98	6.63	13.60	12.20
11	32	3.39	3.25	3.28	10.56	16.80	12.24	2.13	2.34	2.26	6.65	12.10	8.45
12	33	1.51	2.30	1.48	21.60	15.84	27.36	0.99	1.76	0.61	14.20	12.10	11.30
13	34	0.63	1.14	0.69	4.80	10.08	7.92	0.42	0.60	0.37	3.20	5.40	4.25
14	34	0.82	1.70	1.16	7.44	9.60	9.60	0.49	1.15	0.72	4.48	6.50	5.93
15	35	1.14	2.49	1.56	3.12	11.04	4.32	1.07	1.25	0.97	2.92	5.55	2.03
16	35	0.55	1.22	1.34	8.40	13.92	7.44	0.31	0.61	0.60	4.80	6.96	3.30
17	35	1.65	2.10	1.42	9.60	14.16	6.48	0.88	1.36	0.98	5.10	9.20	4.38
18	37	2.05	1.83	2.02	7.68	7.44	7.20	1.46	2.35	1.85	5.50	9.55	6.60
19	39	1.03	2.18	1.08	12.48	17.04	15.60	0.55	1.04	0.45	6.70	8.10	6.50
20	39	1.59	2.43	1.95	7.92	11.28	12.00	0.78	1.45	0.78	3.90	6.70	4.67
21	44	2.30	3.04	2.56	6.96	24.24	19.68	1.98	2.08	1.42	6.00	16.60	10.90
22	47	2.39	2.83	1.86	2.16	6.96	5.76	1.59	1.90	0.96	1.44	4.67	2.99
23	56	2.37	1.70	2.11	5.52	3.12	4.08	1.31	1.09	1.06	3.05	2.00	2.04
24	72	0.80	1.23	1.35	1.68	3.60	3.12	0.78	0.95	0.88	1.65	2.77	2.04

* *Expressed as 11-desoxycorticosterone equivalent.*

We have previously reported a general pattern of daily excretion of 17-ketosteroid involving a maximum output shortly after waking and a decline thereafter to minimal values at night (1, 2, 3). Departures from this pattern are occasioned by stressful activity (2, 3, 6) but where it generally obtains, we would expect "sleep" outputs to be low, the "morning" output highest and the "day" values less than the "morning." Among these 24 sets of collections, 19 exhibit this typical pattern on the hourly calculation, 18 on the gram-creatinine basis. The data of Table 1 show a similar excretion pattern for the neutral reducing lipid; 17 and 19 sets of determinations

show this pattern among the 24-hour rate and gram-creatinine values respectively.

In Table 2 we present the mean output values for the 24 subjects and the percentage increase of the morning and day values over the sleep values. We previously reported approximately a 60 per cent increase of morning over sleep values (5) of 17-ketosteroid calculated on an hourly excretion basis. For these data the increase is 58.1 per cent. The neutral reducing lipids show a mean increase of 47.3 per cent for the same period. The

TABLE 2. THE MEAN URINARY OUTPUT VALUES FOR 17-KETOSTEROID AND NEUTRAL REDUCING LIPID AND THE VARIABILITY THEREOF

	Sleep	Morning		Day		Values of "t" for per cent difference between sleep and		Coefficients of Variation (%)		
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.
	Mg.	Mg.	Per cent increase over sleep	Mg.	Per cent increase over sleep	Morning	Day	Sleep	Morning	Day
Neutral reducing lipid—per 24 hrs.	1.45 ±0.153	2.12 ±0.128	47.3	1.72 ±0.124	18.6	3.40	2.57	51.7	29.6	35.3
17-ketosteroid per 24 hrs.	9.00 ±0.929	13.77 ±1.404	58.1	11.79 ±1.259	31.0	3.14	2.66	50.6	50.0	52.3
Neutral reducing lipid—per Gm. creatinine	0.93 ±0.104	1.31 ±0.104	40.8	0.97 ±0.091	6.4	3.33	1.96	54.8	39.0	44.8
17-ketosteroid per Gm. creatinine	5.70 ±0.637	8.17 ±0.792	43.3	6.45 ±0.629	13.21	3.21	1.34	54.7	47.5	47.8

percentage differences between the sleep and morning values for both 17-ketosteroid and "cortin" are statistically significant on either basis of calculation ($p < 0.01$, column 6). The percentage differences between sleep and day values lie just below the 2 per cent level of confidence ($p = 0.02$ when $t = 2.5$ @ 23 degrees of freedom) when the mg. per 24-hour values are examined; but lie above the 5 per cent level of confidence on a gram-creatinine basis. The data then, on the whole, exhibit significant rises in output for the waking hours and demonstrate a genuine diurnal change. The percentage increase in 17-ketosteroid values for the waking hours is, on the average, higher than the corresponding "cortin" increases (columns 3 and 5) but in these data the differences are not statistically significant. This may in part be due to the rather large variation between men. We have previously observed that each individual tends to excrete 17-ketosteroid at

a fairly characteristic level but that among normal men there is a wide absolute scatter (1, 2). The coefficients of variation listed in Table 2 (columns 8, 9, 10) illustrate this variability. It is interesting that in the morning values and to a lesser extent in the day values, the scatter is decreased for the "cortin" but not particularly for the 17-ketosteroid (cf. columns 9 and 10 with column 8).

In view of the fact that the outputs of both 17-ketosteroid and neutral reducing lipid show the same sort of diurnal rhythm, we have attempted to see if they are correlated. In Table 3 we present the correlation coefficients for the absolute outputs and the percentage and absolute increases. These coefficients were calculated on the mg. per 24-hour output data. The only

TABLE 3. CORRELATION COEFFICIENTS FOR VARIOUS 17-KETOSTEROID AND NEUTRAL REDUCING LIPID DATA

	Period	1. Sleep*	2. Morning*	3. Day*	4. All Values†
A	Reducing lipid vs. 17-ketosteroid absolute outputs	+0.046	+0.612	+0.153	+0.372
	Period	Per cent change, morning over sleep*	Per cent change, day over sleep*	Absolute change in output, morning over sleep*	Absolute change in output, day over sleep*
B	Reducing lipid vs. 17-ketosteroid output changes	+0.024	−0.319	+0.377	−0.214

* D.F. = 23 r @ 5% level of confidence = 0.396
† D.F. = 70 r @ 5% level of confidence = 0.232
r @ 1% level of confidence = 0.302

significant correlation coefficients are those for the absolute output levels for all the data (column A4) and especially for the morning outputs (column A2). It is clear that the significant overall correlation is heavily weighted by the high correlation for the morning outputs. These significant positive correlations indicate that in these men, a relatively high output of 17-ketosteroid is generally accompanied by a high output of "cortin" and vice versa; and that this is especially true in the morning hours. Since this is the period when the values of both 17-ketosteroid and "cortin" tend to be at a maximum, we might expect a significant correlation between the absolute increases of each over the sleep level (column B3). The correlation coefficient of +0.377 actually obtained just fails of significance. Since

no significant correlation for output changes is obtained either on a percentage or absolute basis we can only conclude that the factors causing 17-ketosteroid to change are not necessarily the same which cause the neutral reducing lipid to change. Calculation of possible correlation between output changes on the basis of gram-creatinine output gives similar nonsignificant values.

DISCUSSION

These data clearly indicate significant diurnal changes in both 17-ketosteroid and neutral reducing lipid output. Moreover the diurnal pattern of urinary excretion is similar for each. Nonetheless, the lack of significant correlation between the changes in the two outputs implies independent variation in these changes. We have previously shown (6) that psychomotor stress in normal men evokes an increased excretion of both 17-ketosteroid and "cortin," whereas the administration of glucose evokes an increased output of the latter but not the former. Since the specimens taken during the waking hours include mealtimes, the carbohydrate ingested (or formed from the food) may especially influence the "cortin" produced. The implication that the adrenal cortex may secrete 17-ketosteroid precursors independent of neutral reducing lipid precursors is most interesting. The fact that there is significant positive correlation between all absolute output values (Table 3, A4) implies that the general level of secretion of these precursors goes hand in hand. It is puzzling, therefore, that there is no significant correlation for the absolute sleep values (Table 3, A1) when one is presumably dealing with a basal level of adrenocortical activity. While it is recognized that 17-ketosteroid precursors may be secreted by the testis, they presumably contribute only a fraction of the urinary output and there is no evidence that testis secretion varies diurnally.

Further consideration of the lack of correlation between changes in these two indices of adrenocortical function leads to the consideration that in the human subject different adrenocorticotropins may be involved. The pituitary seems to be essential for an increase of adrenocortical secretion during the alarm reaction (8) and if we consider these diurnal changes as responses to the stresses of daily life a differential evocation of pituitary adrenocorticotropin is suggested. It is interesting to note that Hemphill and Reiss (9) in comparing reducing lipids in adrenals and blood after cold stress and corticotropin administration in animals, obtain different responses. That urinary 17-ketosteroid and corticoids do not go hand in hand has already been shown by Venning and Kazmin (10) for normal individuals and by Venning (11) for pregnant women. Venning's measurements were for glycogenic urinary steroids and these are certainly not identical with the neutral reducing lipids we have measured.

SUMMARY

The urinary output of 17-ketosteroid and neutral reducing lipid was measured simultaneously on aliquots of "sleep," "morning" and "day" samples from 24 normal, healthy men aged 24 to 72 years. The great majority of these subjects showed minimal excretion of both types of substance during the sleep period, maximal output in the morning and less than morning values in the day specimens, when the output is expressed on either a per hour or a per gram of creatinine basis. The mean output increases for both the morning and day excretions are statistically significant. There is a significant correlation between absolute levels of 17-ketosteroid and neutral reducing lipid. Although a similar diurnal pattern of excretion occurs for both types of urinary substance the changes in excretion (on either a percentage or absolute change basis) of each are not significantly correlated. This is taken to indicate the factors evoking urinary 17-ketosteroid increase (or decrease) are not the same as those evoking neutral reducing lipid change.

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EFFECTS OF STARVATION ON SEX HORMONES IN THE MALE

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I. INTRODUCTION

FOR many years there has been considerable interest in the relation of sex hormones to changes in the skin, sebaceous glands, hair, libido, prostatic fluid, breasts and menstruation. An immense amount of research has been completed relative to sex hormones, but much of this work has been carried out on small animals. The question has frequently arisen: "Would the results have been the same in the human?" Thirty-eight months in Japanese Prisoner of War Camps presented excellent opportunities to observe the clinical effects of starvation on the sex hormones of the human. The observations are set forth in this paper, and are compared to some of the results of experiments which have been carried out on small animals by various investigators.

II. CONDITIONS IN JAPANESE PRISON CAMPS

During the few months on Bataan and Corregidor, the slim diet of rice and fish had been cut to half-rations, and finally to quarter-rations. Many of the front-line troops went for days without food. When the Fil-American Forces surrendered during April and May of 1942, many vitamin deficiency diseases were already well established. Then came the "Death March" from Bataan to Camp O'Donnell without food, and with only the little water that could be sneaked from the ditches alongside the road, when the Japanese guards were not watching.

At Camp O'Donnell prisoners died at the rate of several hundred each day. The majority of deaths were among the Filipino troops, because of their proportionately larger numbers, not because of any difference in the treatment of the two groups.

In June 1942 the American prisoners were segregated and taken on a second "Death March" to Cabanatuan, Nueva Ecija. No preparation had been made at the new camp for the reception of the exhausted and starved prisoners. They were crowded into grass shacks, where each man was allotted two by six feet of floor space for sleeping. Many were without blankets and mosquito-nets, and very few had adequate clothing or shoes. Diseases (malaria, dysenteries, vitamin-deficiency diseases and diphtheria)

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were prevalent. Sanitary provisions were completely wanting. Medical care was also lacking, because the doctors and medical corpsmen were as exhausted, starved and sick as the other prisoners, and there was very little medicine.

After a few months in the new camp, all of the prisoners who were still able to walk were forced to work all day in the hot sun on the "Farm."

III. STARVATION DIET

The diet consisted of two to four hundred grams of a very poor grade of dirty rice, and greens (the weeds of carabao wallows). About once a week or less a carabao (water buffalo) was killed for meat. The Japanese took the choice parts, and donated the remains to the American "Mess Shacks" to be cooked into a thin gravy for the nine thousand prisoners. The few prisoners who had been able to smuggle money into camp, and those who got paid for working on the "Farm," were able to purchase bananas, mango beans, camotes (sweet potatoes), and duck eggs in very small quantities, and at very inflated prices. When the pump was working, one canteen of water could be obtained by standing in line for an hour or more.

IV. MORTALITY AND MORBIDITY

The mortality and morbidity were both high. As many as fifty and occasionally more, shrivelled and bloated bodies were daily dropped into common graves. The Japanese were repeatedly asked for medicines, vitamins and additional food, but always gave the same answer: "Mati mati" meaning "After a while."

By December 1942 (eight months in prison camp) more than twenty-four hundred prisoners had died at Cabanatuan Prison Camp. Another twenty-five hundred lay seriously ill on the floors of the makeshift hospital. Most of these patients had lost as much as fifty per cent of their body weight. All of the diseases described in the literature as associated with vitamin deficiency had become prevalent.

V. EVIDENCE OF ANDROGEN SUPPRESSION

Early in starvation there was a universal loss of libido, and an absence of nocturnal emissions. Several months later many of the prisoners complained that their hair was becoming thin. The hair on the head and face became soft, fine and sparse. Where it had been necessary to shave daily prior to imprisonment, shaving once a week was now sufficient. Hairy chests, arms and legs became almost bare. Axillary and pubic hair became thin, with a tendency in some for the hair to assume the feminine distribution.

The skin became thin and loose. It could be picked up from the under-

lying tissue and freely moved about. The sebaceous glands of the face, chest and back became atrophic, and no longer contained comedos. The oiliness of the skin disappeared. Prisoners who had previously been affected with acne vulgaris and seborrheic dermatitis were freed of these conditions.

Some claimed that their testes had atrophied, but this could not be accurately checked, as no measurements had been taken prior to starvation.

VI. EVIDENCE OF ANDROGEN STIMULATION

In December 1943 and the months following, each prisoner received four parcels (eleven pounds each) of Red Cross food and vitamins. The extra food and vitamins made the diet adequate for a period of from two to four months, depending upon the individual and his ability to eat sparingly. The death rate dropped suddenly from three hundred a month to two or three a month. The general health of the camp improved. There were weight gains of from five to twenty pounds.

It was only a matter of several weeks after the diet became normal, that libidos, erections, and nocturnal emissions returned. A few weeks later, prisoners noted hair returning to the bare areas, beards becoming thicker and tougher, sebaceous glands becoming active, and in a few cases the reappearance of acne vulgaris and seborrheic dermatitis.

VII. "TRUE" GYNECOMASTIA

From three to twelve weeks after the diet became adequate, gynecomastia appeared in 300 (six per cent) of the fifty-five hundred prisoners remaining in camp. The ages varied from 18 to 64 years. Fifteen per cent gave a previous history of gynecomastia at puberty. At least fifty per cent were bilateral, although it was usual for one tumor to precede the second by from one to eight weeks. The size varied from 2 to 5 centimeters (average 24 millimeters). Ninety per cent were tender or painful. Three per cent secreted a colostrum-like substance. In only one case did it have the appearance of milk. No definite history of injury was obtained in any of this series. The tumors reached maximum size in from one to nine weeks, and remained stationary until they began to disappear spontaneously (average, four months). None showed any tendency to grow after the size became stabilized. None became malignant during the period of observation.

VIII. FURTHER STARVATION

The Red Cross food and vitamins were largely consumed within two to four months. The diet reverted to rice and greens, supplemented by a rare duck egg, banana, or small portion of mango beans. The diet was

again definitely inadequate, especially in fat and protein. Deficiency diseases and the "castration syndrome" gradually reappeared. Gyneeomastias slowly became painless, and disappeared over a period of from one to twenty-four months (average four months).

IX. LIBERATION—ADEQUATE DIET

Finally after twenty more months of starvation and disease, American food arrived via parachutes just prior to liberation (August and September, 1945). The diet was adequate again. Prisoners gorged themselves. Weight gains of seven to fifteen pounds a week were the rule. Within a few weeks there was evidence that the hormones were being stimulated. A common greeting of the morning became "I'm a man again." Also within a few weeks gynecomastia made its reappearance. It was much more prevalent than it had been after the Red Cross parcels in 1943–44.

Hair gradually became thicker, and in some cases returned to bald areas. Sebaceous glands became hypertrophic and the skin oily. Acne vulgaris and seborrheic dermatitis returned to those previously affected. Thin and fragile fingernails and toenails became thicker.

Many of the prisoners held genuine fears of sterility and impotence, after more than forty months of starvation. However a lapse of two years since liberation has resulted in at least a normal number of pregnancies of the prisoners' wives, and in the normal births of apparently healthy babies.

X. DISCUSSION

It must be obvious to the reader that studies of the androgen-estrogen ratios and levels, and other laboratory determinations were impossible, as there were no facilities. However, an effort has been made to correlate the elinieal observation made of the prisoners, to recent investigations of the effeets of starvation, which have been made largely upon animals.

It has been known for some time that spermatic function of the testes is adversely affected by defieieney of vitamin B_1 or E, and that atrophy of the testes has resulted in some eases.

Pazos and Huggins (1) have shown in animals that complete deprivation of food from four to eleven days eauses a cessation of prostatie fluid due to androgenic deficiency. They believe this is due to a decreased gonadotropin production, and found that they could restore the fluid by injections of testosterone propionate. In their experiments the testes remained capable of androgen produetion for about twenty-one days of starvation.

The symptoms seen in the starving prisoners, described in Section V, are similar to those seen in the eastrated male (2), and are likewise attributed to hypogonadism and androgen suppression.

Hooker and Pfeiffer (3) have produced the "castration syndrome" (atrophy of hair follicles, loss of hair, thinning and loosening of the skin, and atrophy of the sebaceous glands) by treating animals with estrogen. They could protect the animal, however, from the castration syndrome by giving androgen as well as estrogen. It is very probable that the "castration syndrome" seen in the prisoners may have been produced by a relatively increased estrogen as well as a suppression of androgen. The recovery from the castration syndrome following the return of the diet to normal, is believed to be due to the stimulation of androgenic function. Thompson and Heckel (4) demonstrated similar results in the eunuch with large doses of testosterone (gradual increase of hair growth, erections, seminal emissions, and acne).

The three hundred cases of gynecomastia in this series developed after the diet became adequate, and not during starvation.

It has been rather well established that the male mammary duct system is sensitive to excess estrogen (5, 6). It responds with hyperplasia of the rudimentary ducts and periductal connective tissue (gynecomastia). Androgen and estrogen levels are normally maintained by the ability of the liver to inactivate excess (7). However, prolonged starvation impairs the liver (8, 9, 10) so that it cannot inactivate excess estrogen. It then appears that the gynecomastia following starvation is a result of excess estrogen subsequent to liver impairment (11, 12, 13). As the impaired liver gradually recovers its function of inactivating excess estrogen, the androgen-estrogen ratio returns to normal, and the gynecomastia gradually disappears.

SUMMARY

1. The effects of starvation on the sex hormones of the human appear to be the same as or similar to those of the animal.

2. Prolonged inanition produces symptoms (castration syndrome) indicating a decrease in sex hormone production.

3. Subsequent adequate diet causes a recovery from the syndrome, thus indicating a stimulation of the sex hormones.

4. Gynecomastia appeared in six per cent of the prisoners, when the diet became adequate subsequent to prolonged starvation, apparently due to imbalance of the sex hormones.

5. Gynecomastia following relief of starvation disappeared spontaneously, both during further starvation, and after several months of adequate diet.

6. Forty months of severe starvation did not produce the expected impotence or sterility in the recovered prisoners.

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THE RAPID RAT TEST FOR PREGNANCY

THE OVARIAN HYPEREMIA RESPONSE AS A ROUTINE DIAGNOSTIC PROCEDURE*

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SEVERAL authors have described rapid tests for pregnancy based on the hyperemic response of the immature rat ovary to pregnancy urine gonadotropin. Salmon and associates (1) observed that ovarian hyperemia was produced within six hours following a subcutaneous injection of pregnancy urine and suggested this as a test for pregnancy. Kupperman, Greenblatt and Noback (2) reported that the duration of the test could be reduced to two hours provided the urine was injected intraperitoneally.

The accuracy of the two-hour test (excluding observations in ectopic pregnancy) has recently been reported by Kupperman and Greenblatt (3) to be 99.5 per cent in a total of 752 tests. Bunde (4) using either the two-hour test with intraperitoneal injections or the six-hour test with subcutaneous injections, obtained an accuracy of only 84.5 per cent in 108 tests. A test developed by Zondek, Sulman and Black (5) consists of two subcutaneous injections of pregnancy urine at an interval of one hour. The accuracy of this test was 69 per cent at two hours, 92.2 per cent at six hours and 99 per cent at twenty-four hours.

The present report is a summary of the authors' experience with the hyperemia test when used as a routine laboratory test for pregnancy. In no instance was the clinical diagnosis known to the observer at the time the test was performed. The results of the tests have been compared with Aschheim-Zondek tests performed on the same urines. Further observations were made on the relationship of the duration of the test to the intensity of the hyperemic response and the variability in responsiveness of the test animals. An attempt has been made to identify those conditions which facilitate the performance of the test with greatest accuracy.

TECHNIQUE

The technique of Kupperman and Greenblatt (3) was followed in most details except that no attempt was made to restrict the duration of the test to exactly two hours.

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ACCURACY OF THE RAPID RAT TEST AS COMPARED WITH THE A.Z. TEST

In 205 cases it was possible to compare the results of the pregnancy tests with the clinical diagnosis. The accuracy in 194 tests, where either a positive or negative A.Z. test was obtained, is indicated in Table 2. In 11 cases an intermediate-type response was obtained in the A.Z. test and these results are compared with the results of the rapid rat test in Table 3.

TABLE 2. ACCURACY OF THE RAPID RAT TEST AND THE A.Z. TEST IN 194 CASES

Test	Clinical Diagnosis Positive			Clinical Diagnosis Negative			Accuracy for All Tests
	No. of Patients	Correct Diagnosis	Per cent Accuracy	No. of Patients	Correct Diagnosis	Per cent Accuracy	
Rapid Rat	128	126	98.4	66	64	97.0	97.9
Aschheim-Zondek	128	126	98.4	66	66	100.0	99.0

In the group of 128 patients with a clinical diagnosis of pregnancy, an incorrect diagnosis was obtained with both tests in two instances. In no case was an incorrect result given by both tests on the same urine and the results of repeat tests agreed with the clinical diagnosis in every instance.

While no false positive tests were obtained with the A.Z. test, 2 incorrect diagnoses were made in the group of 66 urines from nonpregnant women with the rapid test. The accuracy for the rapid rat test was 97.9 per cent as compared with 99.0 per cent for the A.Z. test.

It has been the authors' experience that a statement of accuracy for any

TABLE 3. RESULTS OF RAPID RAT TEST WITH URINES GIVING INTERMEDIATE-TYPE RESPONSES IN A.Z. TESTS

Clinical Diagnosis	No. of A.Z. Tests with Intermediate Responses	Results of Rat Tests	
		No. Positive	No. Negative
Probable spontaneous abortion	4	0	4
Early pregnancy	1	1	0
Threatened abortion	2	2	0
Incomplete abortion	1	1	0
Early postpartum	1	1	0
Uterine fibromyoma	1	0	1
Bilateral hydrosalpinx and amenorrhea	1	1	0

Fig. 1. Ovaries of animal on right illustrate a positive hyperemic response. The marked reddening of these ovaries is in striking contrast to the pale ovaries of the control animal on the left.

biological pregnancy test requires some qualification. In order to determine the above stated accuracies it was necessary to eliminate those tests in which intermediate-type reactions were obtained with the A.Z. tests. These weak reactions are not uncommon and though the clinical condition of the patient may provide a clue as to the cause for a low chorionic gonadotropin titre, the test result is not a conclusive one. Several conditions which were associated with intermediate-type reactions are listed in Table 3. Similar reactions have occasionally been obtained with urines from postmenopausal and amenorrheic women. When urines which give an intermediate-type reaction in the A.Z. test are used in the rapid test rat the results are variable, in some cases being positive and in others negative. The type of reaction obtained undoubtedly depends in large part upon whether or not there is an adequate amount of the essential gonadotropin in the urine to produce a recognizable hyperemic response.

RELATIONSHIP OF TIME TO THE HYPEREMIC RESPONSE

Zondek and Sulman (6) have emphasized the importance of the time factor in the production of ovarian hyperemia following subcutaneous injections of chorionic gonadotropin. We have attempted to evaluate this factor for tests in which pregnancy urine or chorionic gonadotropin was injected intraperitoneally.

Two dosages (1 I.U. and 20 I.U.) of chorionic gonadotropin were used and tests were allowed to run for intervals of two, four, six and sixteen hours. The response and degree of hyperemia noted in each test animal is tabulated in Table 4. It will be observed that negative responses were obtained in only 3 of the 20 animals used. Two negative responses were obtained in the two-hour group and one in the sixteen-hour group. While all test animals gave a positive response at the end of four hours, the hyperemic response was uniformly more intense in the six-hour group.

In an additional series (Table 5), 2 cc. of pregnancy urine was injected intraperitoneally and the animals were sacrificed at intervals of two, six and sixteen hours. The same dose was administered subcutaneously to a fourth group and the animals were sacrificed at the end of sixteen hours. In the total of 20 animals used, no negative responses were obtained. The least intense hyperemia was observed in the two-hour group, though the heaviest test animal (70 Gm.) of the group showed marked ovarian hyperemia. Approximately the same degree of hyperemia was found in the intraperitoneally and subcutaneously injected animals after sixteen hours.

Following the administration of both chorionic gonadotropin and pregnancy urine, the strongest hyperemic response was obtained most uniformly in the heaviest test-animals (55 to 70 Gm.).

SPECIFICITY OF THE HYPEREMIC RESPONSE

Several types of hormonal preparations were used to determine the specificity of the ovarian hyperemic response. The results, summarized in

TABLE 4. HYPEREMIC RESPONSE TO CHORIONIC GONADOTROPIN AT VARYING INTERVALS OF TIME

Duration of Test	Animal Number	Body Wt. Gm.	Dosage of Chorionic Gonado-tropin* I.U.	Response	Intensity of Hyperemia**
	11	36	1	—	0
	12	45	1	+	I
Two hours	13	60	20	+	II
	14	42	20	—	0
	15	43	20	+	I
	6	42	1	+	I
	7	44	1	+	II
Four hours	8	55	20	+	III
	9	44	20	+	II
	10	42	20	+	III
	1	42	1	+	III
	2	43	1	+	III
Six hours	3	60	20	+	III
	4	42	20	+	III
	5	44	20	+	II
	16	46	1	+	II
	17	36	1	+	I
Sixteen hours	18	60	20	+	III
	19	48	20	—	0
	20	42	20	+	I

* Chorionic gonadotropin obtained through the courtesy of The Upjohn Company, Kalamazoo, Michigan.

** The intensity of the hyperemic response was arbitrarily graded from I to III depending upon the redness of the ovaries.

Table 6, indicate the high degree of responsiveness to both chorionic gonadotropin and the pituitary luteinizing hormone. The positive hyperemic response obtained with the higher dosage of FSH was probably due to the presence of a trace of luteinizing hormone.

DISCUSSION

Our primary interest in the hyperemia response of the rat ovary is whether or not it is sufficiently dependable to be the basis for a rapid, routine test for pregnancy. Although Kupperman and Greenblatt (3) have reported a high degree of accuracy with a two-hour test, others have obtained less gratifying results. Both Bunde (4) and Zondek and his associates (5) have reported relatively low per cent accuracies with both the two and six-hour tests. The latter group of workers, with subcutaneous injections, have found that only the 24-hour hyperemia test has all the accuracy of the older A.Z. test.

TABLE 5. HYPEREMIC RESPONSE TO 2 CC. OF PREGNANCY URINE* AT VARYING INTERVALS OF TIME

Duration of Test	Animal Number	Body Wt. Gm.	Response	Intensity of Hyperemia**
Two hours	21	70	+	III
	22	43	+	II
	23	45	+	I
	24	44	+	I
	25	42	+	I
Six hours	31	46	+	III
	32	46	+	III
	33	44	+	II
	34	40	+	II
	35	42	+	II
Sixteen hours (Intraperitoneal injection)	36	42	+	III
	37	46	+	III
	38	64	+	III
	39	42	+	II
	40	44	+	II
Sixteen hours (Subcutaneous injection)	41	44	+	II
	42	46	+	III
	43	45	+	II
	44	42	+	III
	45	65	+	III

* In tests on the immature mouse, the potency of this urine was found to be 1000 M.U. per 1 cc. This concentration is comparable to that found in urines obtained at the peak of chorionic gonadotropin excretion during the first trimester of pregnancy.

** See explanation, Table 4.

We have altered the technique of Kupperman and Greenblatt to the extent that the test is allowed to run usually between four and six hours rather than confining it to a two-hour interval. The longer intervals provide additional time for the development of hyperemia and at the same time permit the results to be reported on the same day the specimen is received. Following this procedure, an accuracy (97.9 per cent) was obtained which compared favorably with that of the Aschheim-Zondek test.

It appears highly probable that different strains of rats may respond differently to chorionic gonadotropin and that this may account in part for the diverse results reported by different authors. The strain of rats used by Bunde (4) showed a great deal more variability in response and were apparently less responsive to chorionic gonadotropin than that used in the present investigation. This is well illustrated by the fact that no animal in Bunde's series responded to 9.9 I.U. of chorionic gonadotropin and only 2 of 5 animals responded to the injection of 50 I.U. In the present study, only 1 rat in 8 failed to respond to 1.0 I.U. of chorionic gonadotropin. It would be of considerable importance to those contemplating the use of the hyperemia test to determine the responsiveness of their test animals. In the event that marked variability or refractoriness is found, a different source of animals should be sought.

TABLE 6. HYPEREMIC RESPONSE OF THE OVARY OF THE IMMATURE RAT TO HORMONE EXTRACTS AND NONPREGNANCY URINE

Type of Extract or Urine	Dosage	Hyperemic Response
Follicle stimulating hormone (FSH)*	40 R.U.	Negative
	200 R.U.	Positive
Luteinizing hormone (LH)*	1 R.U.	Negative
	5 R.U.	Positive
Chorionic gonadotropin	1 R.U.	Positive
Nonpregnancy urine: Amenorrhea	25 cc.	Negative
14th day of cycle	2 cc.	Positive
16th day of cycle	2 cc.	Negative

* Fractionated pituitary extracts obtained through the courtesy of Difco Laboratories, Detroit, Michigan. In tests on the immature mouse, the FSH preparation was found to contain traces of LH.

As Zondek and Sulman (6) have recently emphasized, the intensity of the hyperemia response is related to the duration of the test. This relation-

ship was more apparent in the series (Table 4) where relatively small amounts (1 or 20 I.U.) of chorionic gonadotropin were administered. Poorer responses were obtained at two hours than at four or six hours. The response at sixteen hours was little better than at two hours. In view of the likelihood that some urines with a low concentration of gonadotropin will be encountered, it would appear to be desirable to allow at least four hours for routine tests.

It is undoubtedly true, as Zondek and associates (5) suggest, that the hyperemia test is of less value than the A.Z. test in cases of disturbed pregnancy, i.e., ruptured ectopic pregnancy and threatened, incomplete or missed abortion. A low concentration of chorionic gonadotropin, which is not infrequently associated with these conditions, can be detected in the routine A.Z. test and is of diagnostic significance. On the other hand, the hyperemia test may be either positive or negative. In either event, the result is noncontributory or misleading from the clinicians' standpoint. In other instances a doubtful hyperemic response may be obtained, necessitating an inconclusive report.

Another disadvantage of the hyperemia test should be emphasized. Although Kupperman and Greenblatt (3) tested urines from nonpregnant women at different times of the cycle and obtained only negative results, Farris (7) has reported obtaining positive hyperemic responses at about mid-cycle. As indicated in Table 6, the pituitary luteinizing hormone induces ovarian hyperemia at relatively low concentrations. At that time in the cycle when the gonadotropin concentration is high, one might expect to obtain a positive hyperemic response. This might lead to a false diagnosis of pregnancy in some instances where the cycles are markedly irregular.

SUMMARY

The application of the hyperemia response of the rat ovary to chorionic gonadotropin as a routine test for pregnancy is discussed. In 205 tests, an accuracy of 97.9 per cent was obtained as compared with an accuracy of 99.0 per cent for the Aschheim-Zondek test. The tests upon which this accuracy was determined were exclusive of 11 tests which gave incompletely positive A.Z. reactions. Such incomplete reactions are not uncommonly obtained in cases of disturbed pregnancy, such as threatened or incomplete abortion. In these cases the hyperemia test may be positive, negative or inconclusive, depending upon the concentration of chorionic gonadotropin in the urine. The hyperemia test is therefore of little diagnostic value in these conditions.

In experiments with chorionic gonadotropin and pregnancy urine, it was found that the hyperemia was uniformly more intense after an interval of four or six hours than after two hours. There appeared to be no advantage

to an interval of sixteen hours, regardless of whether the urine was injected subcutaneously or intraperitoneally.

Tests with several hormonal preparations indicated a high degree of effectiveness of both chorionic gonadotropin and pituitary luteinizing hormone in producing ovarian hyperemia.

The test animals used in this study were highly responsive to chorionic gonadotropin and showed little variability in response. It is suggested that the lower accuracy experienced by other workers may be due to a relative refractoriness of some strains of rats.

The rapidity and simplicity of the hyperemia test, together with its high degree of accuracy, make it a valuable adjunct to other biological diagnostic procedures.

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subjects. No apparent explanation could be offered for this finding. He had previously had assays performed in the laboratory of Dr. Fuller Albright (4) and excessive quantities of cortin were found by bioassay on one occasion. Two subjects in the obese group, A. S. and K. M., excreted large amounts of formaldehyde-liberating compounds, but this could not be confirmed on repeated assays. We have concluded that these isolated findings could represent technical errors early in our experience.

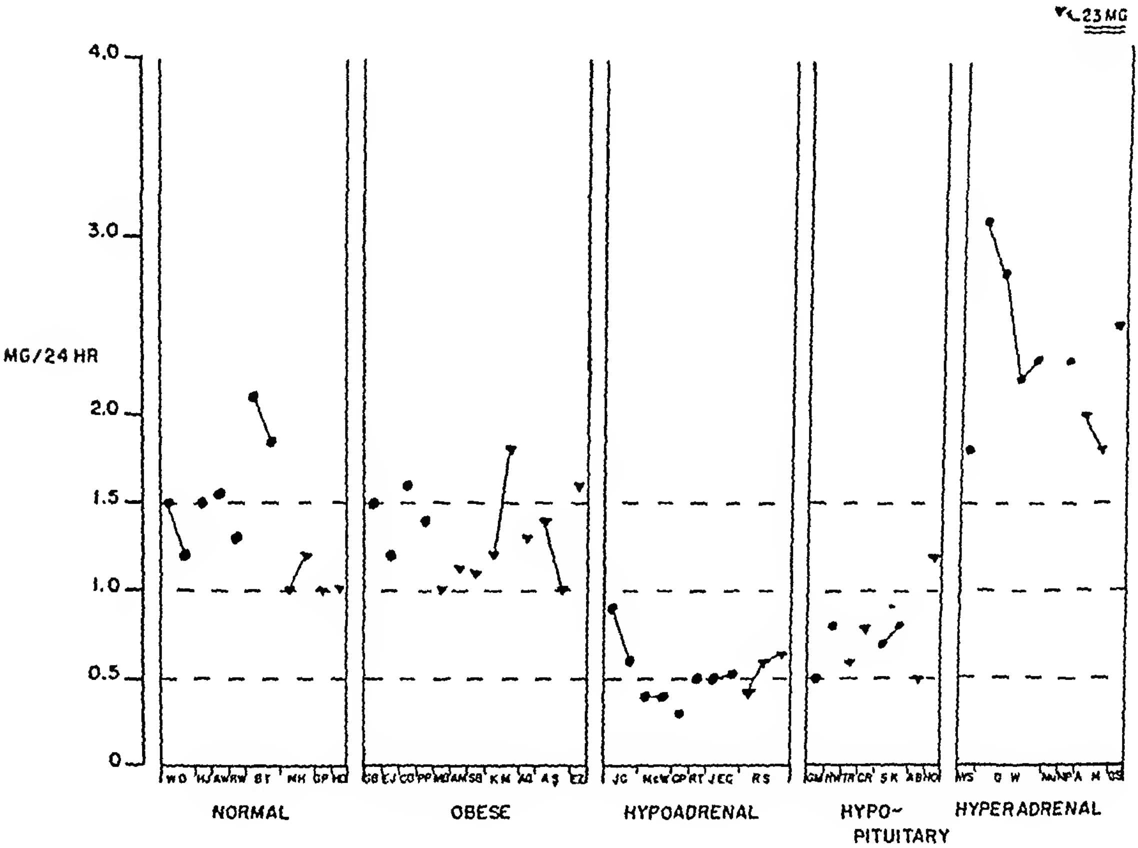

FIG. 1. Excretion of formaldehydogenic steroids in normal and in obese subjects, and in patients with adrenal and pituitary disease. Assays on male subjects are represented by dots and on female subjects by triangles. Multiple assays on the same patient are connected by a solid line. (The broken lines merely help to compare the values in the various columns.)

Hypertension in itself was not observed to be associated with excessive excretion of formaldehyde-liberating material, according to our limited number of observations. The possibility remains that other steroids of adrenal origin, not measured by this technique, are implicated in this disorder.

Excretion of formaldehydogenic steroids in adrenal and pituitary disease. Twelve assays have been performed on the urine from six patients with Addison's disease and the results are presented in Table 2. Except for

the first assay on J. C., all values were between 0.3 and 0.65 mg. per day. These patients all presented the pathognomonic features of the disease. At the time of assay they were all in relatively good nutritional and metabolic state.

The patients with Cushing's syndrome require more explanation. E. M. was a young woman who displayed a full-blown picture of this disease, with

TABLE 2. EXCRETION OF FORMALDEHYDOGENIC STEROIDS IN ADRENAL AND PITUITARY DISEASE

Subject	Sex	mg./day	Subject	Sex	mg./day	Subject	Sex	mg./day
A. Addison's Disease			B. Cushing's Syndrome			E. Panhypopituitarism		
J.C.	M	0.9	E.M.	F	23.0	C.R.	F	0.8
J.C.	M	0.6	G.W.	M	3.1–2.8	T.R.	F	0.6
McW.	M	0.4	G.W.	M	2.2	G.M.	M	0.5
McW.	M	0.4	G.W.	M	2.3	H.W.	M	0.8
C.P.	M	0.3	W.S.	M	1.8	S.K.	M	0.7
R.T.	M	0.5	C.McL.	F	1.2 (in remission)	S.K.	M	0.8
J.Co.	M	0.5				A.B.	F	0.5
J.Co.	M	0.5				H.O.	F	1.2
J.Co.	M	0.4						
R.S.	F	0.4	C. Suspected Hyperadrenocorticism			F. Acromegaly		
R.S.	F	0.6						
R.S.	F	0.65				H.	F	1.3
			M.P.	M	2.3			
			A.M.	F	2.0			
			A.M.	F	1.8			
			C.M.S.	F	2.5			
			D. Adrenogenital Syndrome					
			G. (post-operative)	F	1.5			

amenorrhea, hirsutism, obesity, cutaneous striae, disturbed carbohydrate metabolism and hypertension. The urine for only one day was available for assay; it contained 23 mg. of formaldehydogenic steroids. The patients G. W. and W. S. present an interesting variant of the syndrome. Each showed the facies of Cushing's disease with a striking distribution of fat about the face and neck. A moderate amount of hypertension was present in each, but carbohydrate metabolism, as measured by glucose tolerance curves and glucose-insulin tolerance curves, was entirely normal. Cutaneous striae were present in G. W., but not in W. S. Osteoporosis was not

severe in either individual. It seems possible that in these two patients the steroids which are active in maintaining blood pressure and electrolyte metabolism were formed to a greater extent than were those that are active in carbohydrate metabolism. This thesis is consistent with the results obtained for excretion of formaldehydogenic steroids, which in each patient was only moderately elevated.

M. P. was suspected of having hyperactivity of the adrenals because of

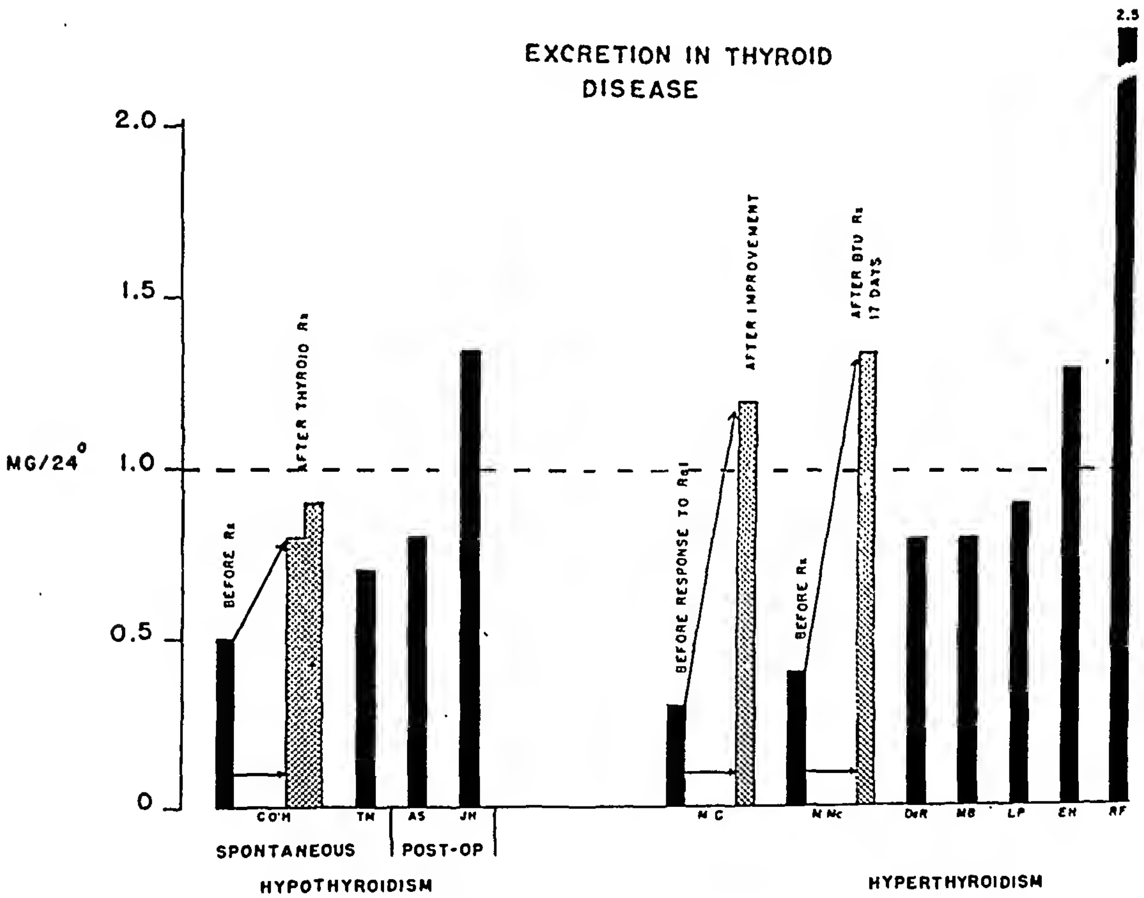

FIG. 2. Note that in both hypothyroidism and hyperthyroidism the excretion of formaldehydogenic steroids tended to be less than 1.0 mg. per day. M. C. was treated with radioactive iodine (I 131). Repeated studies on this patient are shown in Fig. 3. Patient M. Mc. was treated with 6-n-butylthiouracil (BTU).

cutaneous striae, obesity and slight impairment of carbohydrate tolerance. Formaldehydogenic steroid excretion on one occasion was found to be 2.5 mg. per day. A. M. and C. M. S. were also suspected of adrenal hyperactivity because of amenorrhea, changes in fat distribution and slight hirsutism. These patients showed impaired glucose tolerance of the insulin-resistant type. Hypertension was also present. Excretion of formaldehydogenic steroids seemed to be elevated in these two patients.

Formaldehydogenic steroids were excreted in the urine of one patient in normal amounts; C. McL. had had Cushing's syndrome at puberty, but she had undergone a spontaneous and persistent remission. A normal value was also obtained in M.G., a patient with the adrenogenital syndrome, who one year previously had had a masculinizing tumor of the right adrenal removed.

Studies of the excretion of formaldehydogenic steroids have been made

TABLE 3. EXCRETION OF FORMALDEHYDOGENIC STEROIDS IN THYROID DISEASE

Subject	Sex	mg./day
	A. Spontaneous Myxedema	
T.M.	F	0.7
C.O'H.	F	0.5
	B. Postoperative Hypothyroidism	
A.S.	F	0.8
J.H.	F	1.4
	C. Hyperthyroidism	
M.C.*	F	.0.3
M.McL.	F	0.4
DeR	M	0.8
DeR.	M	0.8
M.B.	F	0.8
L.P.	F	0.9
E.H.	F	1.3
E.S.	F	0.8
R.F.	M	2.5

* The value recorded occurred during the most severe stage of her disease. See Fig. 3 for additional assays on this patient.

on seven cases of panhypopituitarism. In two patients (C. R. and T. R.) the cause of the disease was postpartum necrosis of the pituitary. Two individuals suffered from chromophobe adenoma (G. M. and H. W.) and the etiology was obscure in three patients (S. K., A. B. and H. O.). The values which were obtained were intermediate between the group with Addison's disease and the normal subjects. In only one individual, H. O., was the excretion within the normal range. No abnormality of excretion of formaldehydogenic steroids was found in the single case of acromegaly.

Excretion of formaldehydogenic steroids in thyroid disease. The results of formaldehydogenic steroid assays on patients with thyroid disease are presented in Table 3 and in Figure 2. Both hypothyroidism and hyperthy-

roidism tended to be associated with decreased amounts of urinary formaldehydogenic steroids. The excretion was more depressed in two cases of spontaneous myxedema than in the two cases of postoperative hypothyroidism. In one patient with spontaneous myxedema excretion of formaldehydogenic steroids returned almost to normal following treatment with desiccated thyroid.

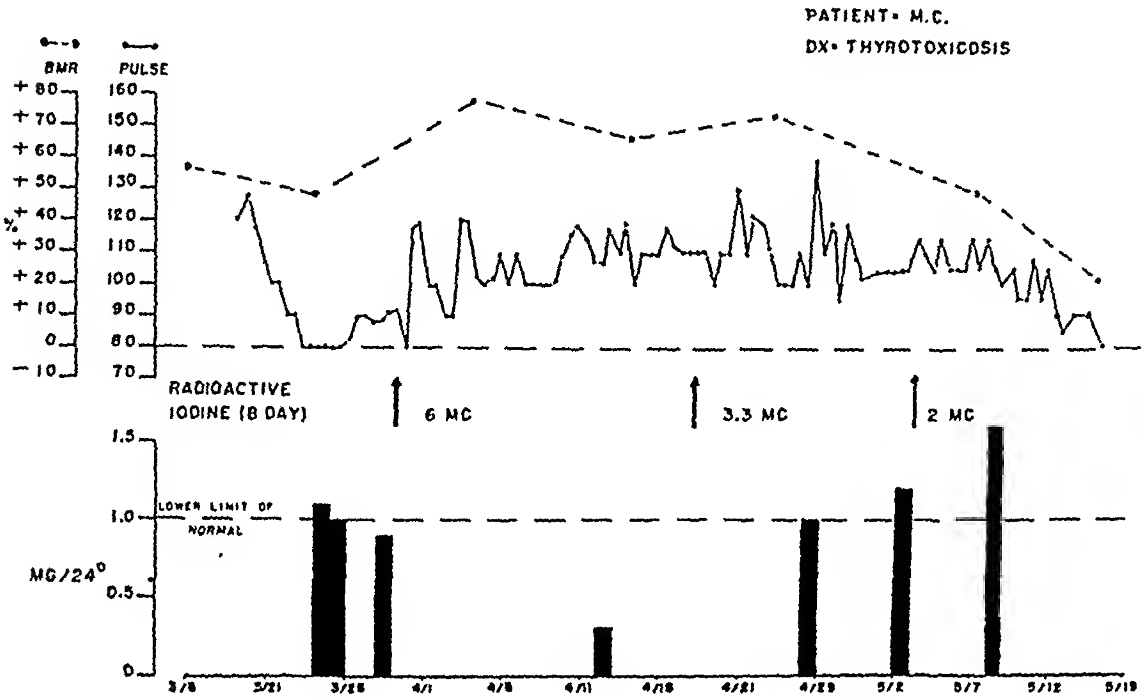

FIG. 3. Excretion of formaldehydogenic steroids in hyperthyroidism treated with radioactive iodine. Note the exacerbation in the disease as evidenced by tachycardia (as well as many other manifestations) produced by the first dose of radioactive iodine.

In two patients with hyperthyroidism the excretion of formaldehydogenic steroids was decreased to levels comparable to that of Addison's disease. In four other individuals the excretion was lower than normal, while in two subjects, normal or excessive excretion of formaldehydogenic steroids was found. In the two patients with the lowest excretion of formaldehydogenic steroids, response to specific treatment (radioactive iodine in M. C. and 6-n-butylthiouracil in M. McC.) was followed by the excretion of normal quantities of formaldehydogenic steroids in the urine.

One of these individuals with hyperthyroidism, M. C., was studied in greater detail and the results are presented in Figure 3. She was a Chinese girl, aged 23 years, who entered the Boston City Hospital with a large thyroid gland and signs of moderately severe hypermetabolism. Improvement followed sedation, bed rest and a good diet with vitamin supplements. Excretion of formaldehydogenic steroids at this time was 1.2, 1.0

and 0.9 mg. per day on successive assays. A severe exacerbation of her disease followed the administration of 6 millicuries of radioactive iodine (I 131). She developed marked tachycardia, palpitation, dyspnea, restlessness, edema of the ankles, weakness and tightness in the neck. There was also an increase in the basal metabolic rate. During this period of mild thyroid storm the excretion of formaldehydogenic steroids decreased to

TABLE 4. EXCRETION OF FORMALDEHYDOGENIC STEROIDS IN SCURVY

Date	mg./day	Remarks
	Patient 1, W.C., Male aged 64 years.	
4 Feb. 1947	0.85	Prior to treatment Vitamin C, 1 Gm. per day by mouth begun on Feb. 6, 1947.
16 Feb. 1947	0.5	
23 Feb. 1947	0.8	
3 Mar. 1947	1.8	
11 Mar. 1947	5.0	
29 Mar. 1947	2.9	
30 Mar. 1947	2.8	
	Patient 2, M.McC., Female aged 64 years.	
6 Mar. 1947	1.0	Prior to treatment Vitamin C, 1 Gm. per day by mouth begun on Mar. 7, 1947
12 Mar. 1947	0.4	
11 Apr. 1947	1.0	
14 Apr. 1947	0.8	
22 Apr. 1947	0.9	
	Patient 3, J. McK., Male aged 30 years.	
2 May 1947	0.4	Treatment started before assay.
6 May 1947	0.2	(see Figure 5)
9 May 1947	0.3	
11 May 1947	0.4	
16 May 1947	1.0	

0.3 mg. per day (checked). When the therapeutic effect of radioactive iodine became evident, formaldehydogenic steroids reappeared in normal amounts in the urine.

Excretion of formaldehydogenic steroids in vitamin C deficiency. Because of the high concentration of vitamin C in the adrenal and the recent chemical (5) and physiological studies (6) suggesting an important role in adrenal function, we have studied the excretion of formaldehydogenic steroids in three cases of clinical scurvy. All three patients had a dietary history suggesting grossly inadequate vitamin C intake; W. C. because of

a self-imposed diet for peptic ulcer, M. McC. because of poverty and poor food habits and J. McK. because of severe alcoholism. Deficiencies of other vitamins undoubtedly existed, but their manifestations were not definite. Subcutaneous ecchymoses and perifollicular hemorrhages were present on

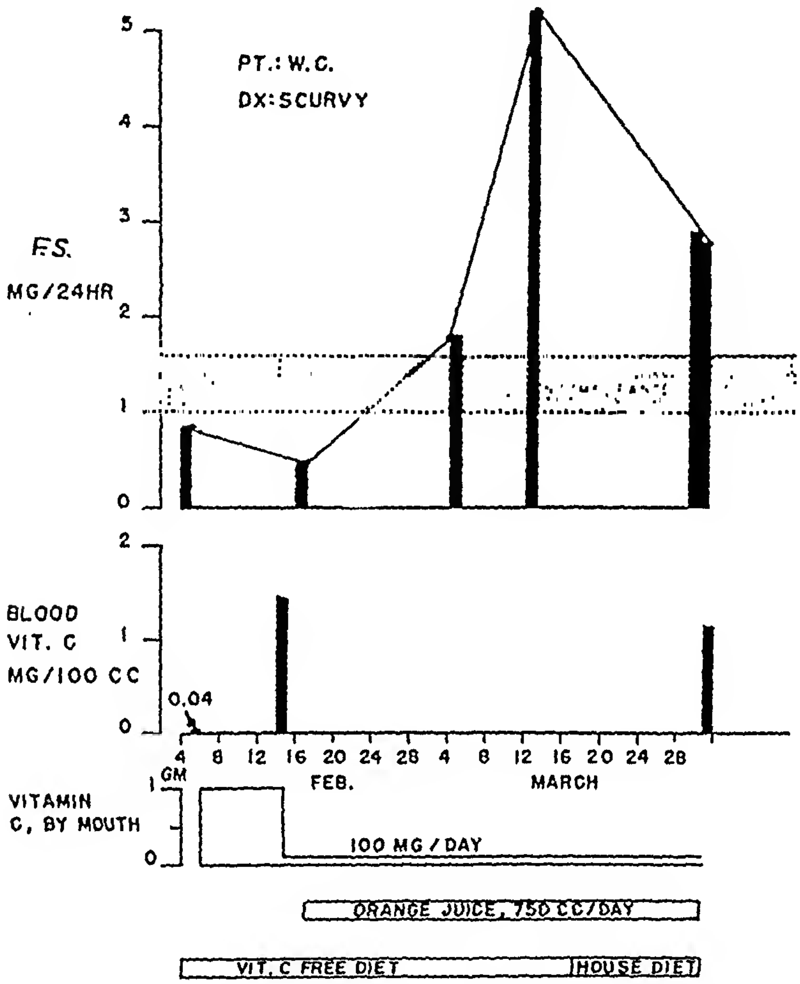

FIG. 4. Excretion in urine of formaldehydogenic steroids (F. S.) in scurvy, patient 1, W. C.

the legs of all three patients when they entered the Boston City Hospital. The tourniquet test was positive in each case. Analyses of the vitamin C content of whole blood in M. McC. and W. C. indicated that less than 0.06 mg. per 100 cc. was present.

Excretion of formaldehydogenic steroids in W. C. before treatment was begun was 0.9 mg. per day. Vitamin C was then administered in a dose of 1 gram a day. Eleven days later the excretion of formaldehydogenic steroids was 0.5 mg. per day and five days thereafter it was 0.8. Later, excessive amounts of formaldehydogenic steroids were found in the urine. The results are presented in Table 4 and Figure 4.

The results of assays on M. McC. are also presented in Table 4. The excretion of formaldehydogenic steroids prior to treatment was 1.0 mg. per day. Following 1 gram of vitamin C daily for 6 days, the excretion of formaldehydogenic steroids had decreased to 0.4 mg. One month later the excretion had risen to more nearly normal levels. No hypersecretory phase was noted, perhaps because of the unequal spacing of the urinary assays.

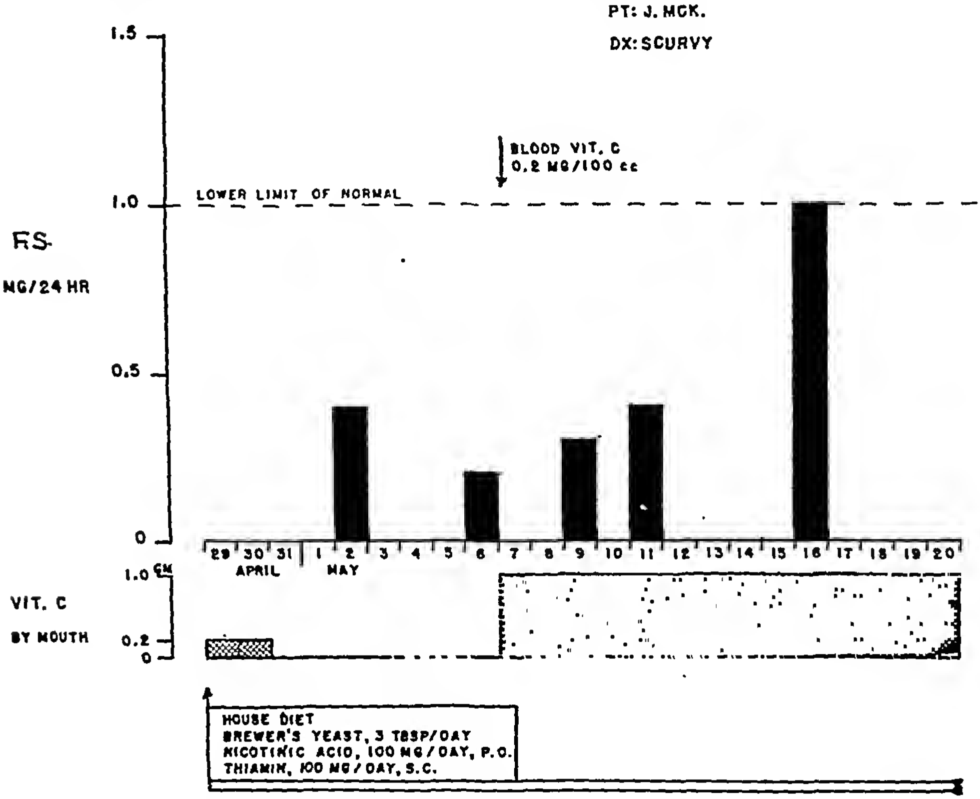

Fig. 5. Excretion in the urine of formaldehydogenic steroids (F. S.) in scurvy, patient 3, J. McK.

The third patient, J. McK., was a man, aged 30, who had been on a long alcoholic debauch and he had eaten very irregularly for several months. Treatment with vitamin C had been started before studies of formaldehydogenic steroids could be started. Subsequent assays of urinary formaldehydogenic steroids are presented in Figure 5 and Table 4. On four separate occasions excretion of formaldehydogenic steroids was below 0.4 mg. per day. It was not possible to make extended observations on this patient. Despite the low excretion of formaldehydogenic steroids in the urine, there were no symptoms or laboratory findings suggesting adrenal insufficiency; in fact the patient showed remarkable improvement.

DISCUSSION

In patients with adrenal disease the quantity of formaldehydogenic steroids excreted in the urine is consistent with the known pathologic physiology of these conditions and confirms reports using bioassay (7) and chemical assay measuring the reducing power of urinary steroids (8, 9, 10). It was found that patients with Addison's disease excreted significantly less formaldehyde-liberating compounds than did normal subjects. The fact that such patients continued to excrete small amounts of these substances warrants consideration of an extra adrenal source for a portion of the material which was measured. An analogous situation has been found to exist with methods measuring the reducing power of urinary steroids (8, 10). The possibility exists, therefore, that there may be variations of this non-adrenal portion which would make interpretation of results difficult. Unfortunately, facilities have not been available to confirm our observations using the bioassay of Venning et al. (3).

The changes in excretion of formaldehydogenic steroids which occur in thyroid disease have received little study. In view of the extensive literature relating adrenal size and function to the activity of the thyroid, this subject warrants careful investigation. Talbot (10) has reported observations on two patients with hypothyroidism. He found that in each patient the excretion of 11-oxysteroids was below normal; following the administration of thyroid to one of them the excretion rose to the lower limit of normal. It has been well established that the excretion of 17-ketosteroids may be very low in myxedema (11).

Hyperthyroidism places the patient under considerable stress and presumably increases the requirement for adrenal cortical hormones. The results from animal experiments make it seem clear that administered thyroid hormone or thyroxine increases the weight of the adrenals in intact animals (12) and increases the sensitivity to potassium toxicity (13). In dogs, at least, adrenal cortical hormone appears to counteract the deleterious effect of thyroxine on nitrogen metabolism (14). Because of the possibility of adrenal exhaustion in "thyroid storm," adrenal extracts have been advocated and used with results which are difficult to interpret (15). The excretion of 17-ketosteroids has been found to be decreased in thyrotoxicosis (11), but little direct evidence has been obtained concerning cortin excretion in this disease. Venning and Browne (7) have reported an isolated observation on a young man with moderately severe hyperthyroidism. Normal excretion of corticoids was found. Our studies of this condition show that there is commonly a slight decrease of excretion of formaldehydogenic steroids, despite the stress imposed by the disease. In some patients with severe hyperthyroidism the cortin excretion was quite low. We have had an opportunity to make assays on one patient, M. C., before,

during and following a mild thyroid storm. A decreased excretion coincided with the most severe phase of the disease.

The reason for the low urinary excretion of formaldehydogenic steroids despite the biological evidence of adrenal hyperactivity remains obscure. It seems possible that the destruction of hormone should be increased by the greatly augmented rate of biological oxidation. In this instance urinary excretion would not represent hormone production.

The observations which we have made on scurvy are also difficult to interpret. Contrary to our expectation, decreased urinary excretion seemed to occur *after*, rather than prior to, vitamin C treatment. McKee, Cobbey and Geiman (16) have recently demonstrated a remarkable impotence of adrenal cortical steroids to raise the level of glycogen in the livers of fasting scorbutic guinea pigs. Whether the administration of vitamin C to patients with scurvy may aid in the formation of a steroid-ascorbic acid complex (5) and in some way prevent excretion remains to be determined.

SUMMARY

The urinary excretion of formaldehydogenic steroids has been estimated by a chemical method based on the liberation of formaldehyde from urinary extracts by treatment with periodic acid. Presumably, this method measures nonglycogenic as well as glycogenic steroids and is, therefore, not considered to be directly comparable with tests of gluconeogenesis. It is very desirable to compare the two types of tests in order to determine which one indicates best the activity of the adrenal glands. It is probable that neither method yields more than a rough indication of the quantity of adrenal hormones manufactured.

In a small number of normal persons the excretion of formaldehydogenic steroids was found to be from 1.0 to 1.6 mg. per 24 hours. In all but one of eleven determinations on 6 patients with Addison's disease the values were less than 0.65 mg. per day.

In 7 patients with panhypopituitarism the excretion was below normal in all but one patient who excreted 1.2 mg. per day. Increased excretion occurred in patients with Cushing's syndrome.

Hypothryoidism and hyperthyroidism were associated with decreased excretion of formaldehydogenic steroids.

The administration of vitamin C to three adult patients with scurvy was associated with a decrease in the excretion of formaldehydrogenic steroids, followed by an increase, which was above normal in one instance.

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RELATION OF OBESITY TO THE FUNCTION OF THE THYROID GLAND, ESPECIALLY AS INDICATED BY THE PROTEIN-BOUND IODINE CONCENTRATION IN THE PLASMA

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IN 1916 Means (1) reported that even in patients with marked obesity the basal metabolic rate was usually normal. Since then much evidence has accumulated (2) to indicate that hypothyroidism does not play an important role in the etiology of obesity, but this interrelationship has not been clarified completely. In individuals with myxedema there does not tend to be much adiposity. For example, Boothby and Sandiford (3) found in 81 per cent of 94 obese patients that the basal metabolic rate was within 10 per cent of normal. Strouse, Wang and Dye (4) observed that the basal metabolic rate of normal persons was similar, per square meter of body surface, to those of subjects who were overweight. Grafe (5) found a definite decrease in the basal metabolic rate in only 3 of 180 patients with marked obesity. In most of the obese subjects whom we studied (6) the basal metabolic rate was normal.

MacKay and Sherrill (7) observed that several months after total thyroidectomy of rats 170 days old there was a much less fat content than in the control animals. Plummer (8) found that only a small proportion of 200 patients with myxedema were significantly obese. Moreover, he observed that when the excess fluid was eliminated, many of the subjects were underweight.

Since the concentration of the protein-bound iodine of the plasma is generally a better indicator of the rate of production of thyroxin than is the test of the basal metabolic rate, determinations of the former, as well as the latter, were made in 24 obese individuals.

MATERIAL AND METHOD

Only markedly obese subjects and ones who had had no iodide for more than six weeks were investigated. None of the patients had any symptoms or physical signs suggesting hypothyroidism. All of the patients were ambulatory and were in good health, except for one with hypertension and

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one with diabetes mellitus. Eighteen of the individuals were in their fourth or fifth decade; 5 were males and 19 were females.

Plasma samples were collected using the precautions outlined by Salter (9). The amount of protein-bound iodine was determined, in 17 cases, by the method of Riggs and Man (10). The plasma from the other 7 patients was analyzed in Dr. Salter's laboratory, using the method which he developed (11). Estimations of the basal metabolic rate were made on approximately the same date as blood was drawn for the iodine determination.

RESULTS

The protein-bound iodine of the plasma was found to range from 1.3 to 8.0γ per 100 cc. of plasma (Fig. 1). The question now arises as to what

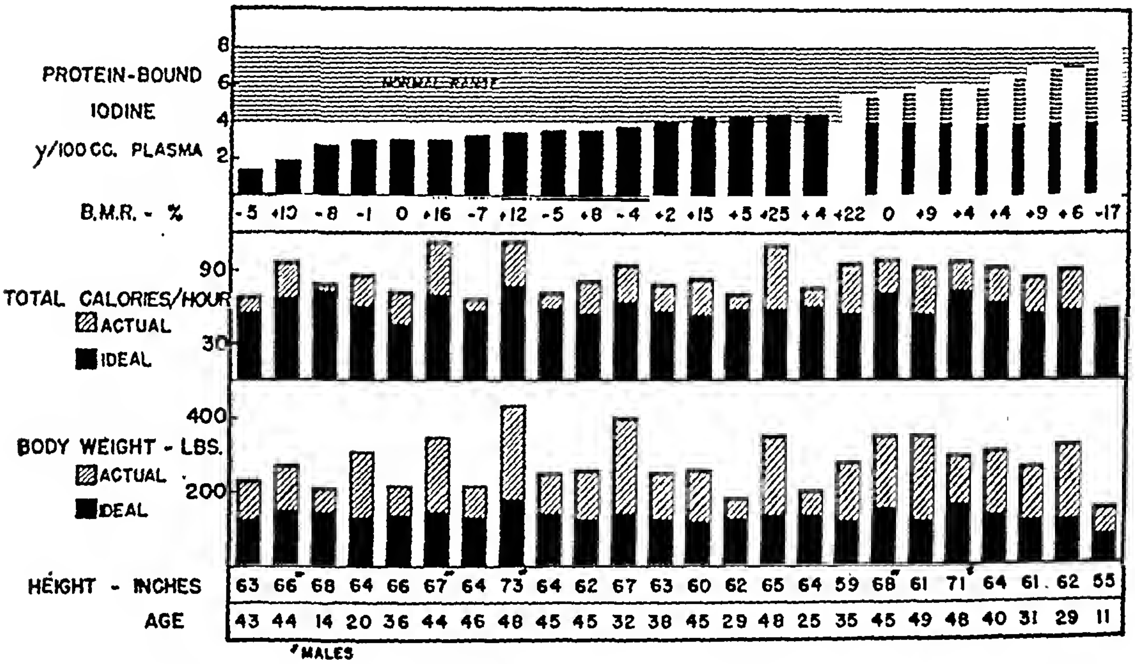

FIG. 1. Note that the concentration of protein-bound iodine was less than 4γ per 100 cc. of plasma in 11 cases. The total oxygen consumption per hour was greater in each instance than that of normal individuals of the same height.

are the limits of the normal range. Relatively few values have been reported on entirely normal individuals, but a moderate number are given for patients with euthyroidism (12). Although exceptions occasionally occur, values from 4 to 8γ per 100 cc. may be considered normal. Winkler (13) has recently presented values for 235 control subjects which ranged from 1 to 15γ per 100 cc. of serum with the majority between 3 and 8γ. Upon comparing the control values obtained by Winkler with the ones which we obtained in the obese subjects, it is observed (Fig. 2) that a greater proportion of the latter group had low concentrations of iodine in

the plasma. In 11 of the 24 patients whom we studied there was less than 4γ; in 5 of the 7 sera analyzed in Dr. Salter's laboratory there was less than 4γ. In only 8 of the 24 patients was the iodine concentration greater than 4.5γ; the highest value was 8.0. There was no significant difference clinically between the patients with hypoiodinemia and the others.

In 18 subjects the basal metabolic rate was in the range between plus 10 and minus 10 per cent. In only one case was it below minus 8, in which instance it was minus 17 per cent. In 5 patients it was between plus 10 and plus 25 per cent, but three of these subjects were dyspneic when they were in the recumbent position.

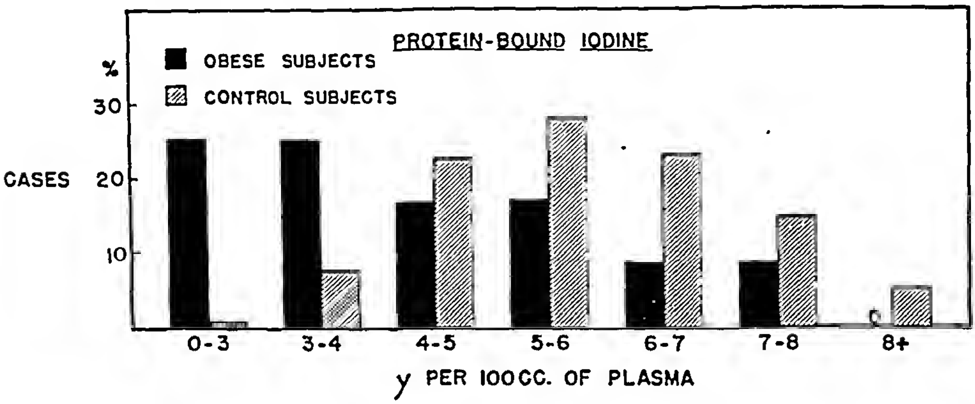

FIG. 2. Note that the protein-bound iodine of the plasma was decreased in a greater proportion of 24 obese subjects than was found by Winkler (13) in 235 control individuals.

As shown in Figure 1, the total number of calories utilized per hour by each patient, in a basal state, was distinctly greater than in individuals of the same height but of ideal weight. There is no correlation of the protein-bound iodine of the plasma with the basal oxygen consumption nor with the amount of obesity or the age of the patient. It can be observed in Figure 1 that there was pronounced obesity in all of the subjects; the average heights were essentially the same as in normal subjects.

DISCUSSION

It is difficult to evaluate the significance of the relatively low concentrations of protein-bound iodine. It is believed that they are technically accurate since (a) most of the determinations were conducted in the same manner as has been found satisfactory in this laboratory for several years, and (b) the results obtained in Dr. Salter's laboratory were comparable to the ones acquired in the Thorndike Laboratory. If hypoiodinemia is of significance in the etiology of obesity, it must play a role in only part of

the patients, although they were similar clinically. Moreover, even though hypoiodinemia had been found in all cases, it might not have been of very much significance etiologically, since patients with myxedema consistently have hypoiodinemia without much obesity. Furthermore, there was not sufficient clinical evidence to diagnose hypothyroidism in any case. Three of the patients were treated for several weeks with 2 grains or more daily of desiccated thyroid without much loss in weight. In 15 obese patients examined postmortem, the thyroid gland was found to be normal macroscopically and microscopically.

Other etiologic factors in obesity are discussed in a separate report (6).

SUMMARY

The functional activity of the thyroid gland in obesity has been studied, with special attention to the concentration of the protein-bound iodine in the plasma. In 11 of 24 obese patients the iodine concentration was less than 4γ per 100 cc., an arbitrary lower limit of normal; in the other 13 cases normal values were obtained. Considering these observations in the light of other findings in obesity, it is concluded that an alteration of thyroid function does not have much etiologic significance in obesity.

ACKNOWLEDGMENT

We are very grateful to Dr. William Salter for the aid that he has given us in these studies.

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STUDIES ON ANTIHORMONE SPECIFICITY WITH PARTICULAR REFERENCE TO GONADOTROPIC THERAPY IN THE FEMALE*

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PROTRACTED administration of extracts containing gonad-stimulating hormones will frequently result in the formation of antihormones (1). With the appearance of these hormone inhibitors two choices present themselves: either to discontinue therapy and await the disappearance of the antihormones, which usually is a matter of 2 to 3 months, or to administer a gonadotropin from another source.

Whether or not a response to another gonadotropic extract in the presence of antihormones can be anticipated has received little clinical consideration. Animal experiments, however, provide leading information and while these studies are too extensive to review here, a good summary in table form is presented by Zondek and Sulman (2). In general, in the rabbit, pregnant mare serum gonadotropin will form antihormones which are specific in their antagonistic action. Sheep pituitary extracts, on the other hand, elicit antigonadotropins which are nonspecific in that they will antagonize the gonadotropic activity of human, ox, horse, pig, rat and rabbit pituitaries, of human chorionic gonadotropin and of pregnant mare serum. Numerous reports are also listed by Zondek and Sulman (2) to show that human chorionic gonadotropin will form antihormones in the rabbit and that these sera are capable of inhibiting human pituitary activity. It is evident from the animal experiments that the source of the gonadotropic extract is a factor in the problem of antihormone specificity. One might, of course, consider the results as being due to extracts which for the most part were not prepared for clinical use. This does not appear to be a determining factor, however, as it has been shown that the clinically used combination of sheep anterior pituitary extract and human chorionic gonadotropin (Synapoidin) will form antigonadotropins in the rabbit and that the serum will inhibit the gonadotropic activity of pregnant mare serum, human chorionic gonadotropin, human pituitary and rat pituitary (3).

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In general, hormones of homologous source do not form hormone antagonists and therefore human chorionic gonadotropin may elicit antihormones in the rabbit but not in man. Furthermore, inhibitory substances against chorionic gonadotropin, human pituitary or sheep pituitary extract do not appear in the serum of postpartum women (4). A recently reported exception must be noted, however, as Segaloff and Parson (5) have presented a case in which inhibitors against chorionic hormone were detected.

Equine gonadotropin (pregnant mare serum) can form antigonadotropins in the human but the inhibitory action appears to be hormone specific in agreement with animal experiments. Rowlands and Spence (6) and later Ostergaard (7) found that anti-PMS serum would not inhibit the gonadotropic activity of human pituitary or human chorionic gonadotropin. Jailer and Leathem (8) could not inhibit the gonadotropic activity of sheep anterior pituitary extract plus human chorionic gonadotropin (Synapoidin) with the serum from a patient showing considerable anti-PMS activity.

Pituitary extracts from animals will form antihormones in man but no information is available to ascertain whether the antagonistic action is specific for the hormone injected or is aspecific to the point of counteracting the secretions from the patient's own pituitary. The current investigation is a beginning study of that problem.

Patients suffering from amenorrhea, sterility or functional menstrual disorders were the cases under study. All were carefully selected and gonadotropins administered because the laboratory findings suggested a deficiency of pituitary gonadotropins. The hormones used were pregnant mare serum (Gonadogen), a combination of sheep anterior pituitary extract and human chorionic gonadotropin (Synapoidin) and human chorionic gonadotropin (Antuitrin-S).[1]

The presence of antigonadotropins in the serum was determined by estimating the degree to which the serum inhibited the ovarian weight increase anticipated with the gonadotropin alone. For these tests, twenty-two day old female rats or mice were used. All animals received the designated dose of gonadotropin and 2 of the 4 littermates received in addition, at another site, a total of 0.9 cc. of the serum to be tested. Injections were made subcutaneously once daily for three days and the animals were killed twenty-four hours after the last injection. The weights of the ovaries and uteri were obtained at autopsy. Pregnant mare serum, human cho-

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[1] Grateful acknowledgment is made to Dr. M. H. Kuizenga of the Upjohn Co. for the Gonadogen, to Dr. D. A. McGinty for the Synapoidin and Antuitrin-S, and to Dr. R. Bates of E. R. Squibb & Sons for the Equine Pituitary Gonadotropin.

rionic gonadotropin, human pituitary, equine pituitary and a combination of sheep anterior pituitary extract plus chorionic gonadotropin were all used to determine serum specificity. The water soluble portion of acetone-dried human pituitaries was used.

The specificity of pregnant mare serum antihormones was studied in 2 cases. Each of these patients had been previously treated with another gonadotropin but the resulting antihormones for pregnant mare serum (PMS) were specific. *Case 1* was a sterile patient who had received 15 R.U. of "Synapoidin" three times weekly during two weeks of each month for a four month period. Pregnant mare serum was then administered during a

TABLE 1. INHIBITORY ACTION OF PREGNANT MARE SERUM ANTIHORMONES IN A STERILE WOMAN (Case 2)

Hormone (Total Dose)	Test Animal. Ovarian Weight (mg.)		Test
	Hormone Only	Hormone and Patient's Serum	
Pregnant Mare Serum (10 I.U.)	11.3	3.4	Positive
"Synapoidin" (3 R.U.)	7.4	9.0	Negative
Human Pituitary (2 mg.)	53.0*	54.0	Negative

* Tested in rats, others in mice.
Ovarian weight of control rats, 12.3 mg.; of mice, 3.1 mg.

two week period (total dose, 2400 I.U.) and her serum tested three weeks later. Her serum exhibited a marked anti-PMS effect but did not alter the action of "Synapoidin." *Case 2* was a sterile patient that had received several courses of injections with chorionic gonadotropin followed by two months of twice weekly injections with pregnant mare serum (total dose, 2000 I.U.). Her serum was tested two months after the last injection and a definite inhibitory action against PMS was demonstrated but the same serum did not alter the ovarian stimulating action of "Synapoidin" or human pituitary in the test animal (Table 1).

Case 3. This patient, (G.), suffered from secondary amenorrhea. She had received extended treatment with the combination of sheep AP and chorionic gonadotropin (Synapoidin) prior to the administration of pregnant mare serum for two months. Shortly thereafter her serum was found to be antagonistic to PMS and to human chorionic gonadotropin but was not inhibitory against "Synapoidin" (Table 2). It would seem that PMS antihormones were not specific in this case and indeed the data do not prevent

this conclusion. However, "Synapoidin" is an extract containing chorionic gonadotropin and sheep anterior pituitary and, as will be presented, its antigonadotropins are not specific. Since this patient received "Synapoidin" for an extended period, either the chorionic gonadotropin which it contained stimulated the development of antihormones or nonspecific antagonists to the sheep protein developed, which were more sensitive to chorionic hormone than to the administered hormone combinations.

The nonspecific antihormones developed following the administration of pituitary extracts are well known and during the course of the clinical studies with "Synapoidin" several rabbits were also injected. The rabbit

TABLE 2. ANTIHORMONE TESTS ON PATIENT G. (CASE 3) AFTER TREATMENT WITH A COMBINATION OF SHEEP ANTERIOR PITUITARY PLUS HUMAN CHORIONIC GONADOTROPIN (SYNAPOIDIN), AND WITH PREGNANT MARE SERUM (GONADOGEN)

Hormone (Total Dose)	Test Animal. Ovarian Weight (mg.)		Test
	Hormone Only	Hormone and Patient's Serum	
Pregnant Mare Serum (10 I.U.)	57	15	Positive
Chorionic Gonadotropin (20 I.U.)	4.5*	2.5*	Positive
"Synapoidin" (3 R.U.)	36	39	Negative

* Tested on mice.

serum was soon found to be antagonistic to these gonadotropins and the finding that human pituitary extract was also antagonized by this serum prompted a more complete study of our clinical material. Patients were treated with "Synapoidin" from two to three times weekly in 15 R.U. (1 cc.) intramuscular doses during the first two weeks of each month. After from two to five months of therapy the sera failed to contain antihormones for "Synapoidin," but the possibility that substances inhibitory to gonadotropins from other sources might be present, while unlikely, was not eliminated. Therefore, a number of these sera were also tested against human chorionic gonadotropin, pregnant mare serum, equine pituitary and human pituitary and with but one exception were found to be negative (Table 3). The exception was a patient tested after three months of treatment, during which time the "Synapoidin" did not form hormones against itself but did elicit the formation of substances capable of antagonizing the action of chorionic gonadotropin. Although it is not possible to say which of the components of "Synapoidin" was responsible for this antihormone

development it does seem logical to assume that the sheep protein formed nonspecific substances which were more potent against chorionic gonadotropin. A similar situation occurred in the previously discussed *case 8* in which PMS was also administered. It is apparent that PMS is not needed to stimulate the development of antihormones for chorionic gonadotropin when "Synapoidin" is administered. The clinical response was clearly demonstrated in cases of functional menstrual disorders which, for the most part, were restored to normality.

After six months of treatment with "Synapoidin" several patients developed substances in their serum which were capable of reducing the

TABLE 3. ANTIHORMONE TESTS WITH SERA FROM PATIENTS TREATED FOR TWO TO FIVE MONTHS, WITH A COMBINATION OF SHEEP ANTERIOR PITUITARY AND HUMAN CHORIONIC GONADOTROPIN (SYNAPOIDIN)

Hormone	No. of Patients Tested	No. of Positive Tests	Test Animal. Ovarian Weight (mg.)		Test
			Hormone Only	Hormone and Patient's Serum	
"Synapoidin"	12	0	42	47	Negative
Chorionic Gonadotropin	6	1	42*	23*	Positive
Pregnant Mare Serum	3	0	39	47	Negative
Equine Pituitary	4	0	30	30	Negative
Human Pituitary	3	0	45	46	Negative

* Data of the one positive test. Others are averages of all tests.

effectiveness of these gonadotropins. In 4 cases the presence of antihormones was reflected by the failure of the patient to respond to previously successful therapy and by the antihormone tests which were positive for the gonadotropic material administered. Furthermore, the partially nonspecific nature of these antigonadotropins was shown by the inhibitory action of the serum against human chorionic gonadotropin and pregnant mare serum but the lack of such action against human pituitary (Table 4).

These studies recall the antihormonic tendencies of gonadotropins with the general exception of chorionic gonadotropin. When the hormone source is pregnant mare serum the antihormones which develop are specific and consequently a clinical response could be anticipated with a pituitary extract in the presence of anti-PMS serum. The extract would possibly have to be from a source other than horse pituitary. "Synapoidin" while showing no evidence of antihormone formation for the first six months of therapy in the doses used may, on a rare occasion, exhibit antichorionic

hormone action during this time. More extended use of "Synapoidin" can elicit nonspecific antihormone formation, and a response to pregnant mare serum hormone in the presence of "anti-Synapoidin" serum would not be anticipated. Unlike the animal experiments, human "anti-Synapoidin" sera do not inhibit the gonadotropic action of human pituitary. It is necessary to emphasize, however, that continuous administration of this gonadotropin might form substances antagonistic to human pituitary but it is apparent that antihormone formation can be detected before such action is possible and serves as a definite reason to discontinue this

TABLE 4. INHIBITORY ACTION OF HUMAN "ANTI-SYNAPOIDIN" SERUM

Hormone	No. of Patients Tested	No. of Positive Tests	Test Animal. Ovarian Weight (mg.)		Test
			Hormone Only	Hormone and Patient's Serum	
"Synapoidin"*	4	4	57	24	Positive
Chorionic Gonadotropin	2	2	39	18	Positive
Pregnant Mare Serum	4	3	38	16	Positive
Human Pituitary	3	0	36	39	Negative

* Sheep anterior pituitary and human chorionic gonadotropin.

therapy. These data further emphasize the importance of antihormone tests in patients receiving extended gonadotropin therapy.

SUMMARY

Further evidence is presented to support the contention that antihormones which develop following the administration of pregnant mare serum are hormone specific. On the other hand, patients who develop antihormones for a combination of human chorionic gonadotropin and sheep anterior pituitary extract (Synapoidin), have sera of a partially nonspecific nature. These sera will counteract the activity of chorionic gonadotropin and pregnant mare serum but will not affect that of human pituitary. In general, if the sera of patients receiving "Synapoidin" therapy fail to show the development of antihormones for the administered extract, they will also fail to show antagonism to gonadotropins from other sources. It appears that pregnant mare serum therapy would be to no avail in the presence of the formation of "anti-Synapoidin" serum by the patient; but that "Synapoidin" therapy would be effective in the presence of anti-PMS serum.

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ANNOUNCEMENT OF THE 1948 MEETING OF THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS

The Thirtieth Annual Meeting of the Association for the Study of Internal Secretions will be held in the Palmer House, Chicago, Illinois, June 18 and 19, 1948.

The scientific sessions will be held in the Red Lacquer Room and registration will be on the fourth floor just outside the Red Lacquer Room. The Annual Dinner will be held in the same room on Friday, June 18th at 7 P. M. and will be preceded by a cocktail party, the location of which will be announced later.

All members of the Association who plan to attend the Thirtieth Meeting are urged to make their reservations at once with the Palmer House, stating the time of arrival and how long they plan to remain in Chicago.

The program of the meeting will appear in either the April or May issue of the Journal, and the abstracts of papers presented at the annual meeting will be published in the July issue.

ANNOUNCEMENT OF THE 1948 LAURENTIAN HORMONE CONFERENCE

The Laurentian Hormone Conference of the A.A.A.S. will meet in 1948 at the Forest Hills Hotel, Franconia, New Hampshire, from September 13th to 18th inclusive. The program (which will be published in full at a later date) will consist of four sections:

I. The metabolism of steroid hormones *in vivo* and *in vitro*.
II. Thyroid physiology and function.
III. The role of hormones in tissue and body metabolism.
IV. Hypothalamic neuro-humoral relationships.

Because of limited accommodations, attendance is by invitation, but the Committee on Arrangements will receive applications for membership until June 15, 1948. Applications should be addressed to: Dr. Gregory Pincus, Chairman, Committee on Arrangements, 222 Maple Avenue, Shrewsbury, Massachusetts.

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THE INTERSTITIAL TISSUE OF A HUMAN HERMAPHRODITE*

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THE site of production of the sex hormones in human males is still a disputed question. In a recent contribution by Witschi and Mengert (1) evidence is presented supporting the contention that the sustentacular cells of the seminal tubules (so-called cells of Sertoli) are the producers of the female sex hormones. In a patient of feminine appearance, without ovaries, but with large testes, the urine contained a considerable amount of estrogens. After removal of the sex glands, the estrogenic substances disappeared. A histologic study proved the complete absence of ovarian cortex and an abortive condition of spermatogenesis. The interstitial cells (of Leydig) were small and did not give the impression of much activity. On the other hand, the sustentacular cells were highly developed, and gave the appearance of intense secretory activity. The morphologic homology of the ovarian granulosa and the sustentacular cells of the testis was stressed, and the origin of the female sex hormones of this patient was attributed to the sustentacular cells.

During the last few years, one of us (Van Campenhout (2, 3, 4, 5)) became interested in the neural relationships of the interstitial tissue in human sex glands, and presented new evidence in favor of the interstitial nature of sympathicotropic cells described by Berger (6). As in human testes and ovaries, intimate connections between interstitial cells of Leydig and nerve

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fibers were also found in the testes of some primates. According to these observations, neuro-epithelial complexes are present in the gonads of both sexes. Since the interstitial cells of the testes and the cells of the theca interna of the ovary are homologous, a similar function might reasonably be expected to exist in these gonads. It was suggested that the nervous

FIG. 1. Part of albuginea from section through hermaphrodite gonad. Nerve trunk (n.t.) in close association with a group of interstitial cells (i.c.). Some nerve fibers pass in between these cells. Stained by the trichromic method of Masson.

stimuli might modify the normal secretion of the homologous elements of the gonads so as to give rise to different hormones.

It was, therefore, decided to make a renewed study of the sections of the sex glands removed from the patient and to test, in this possibly crucial case, the validity of the last mentioned hypothesis. Absence of neuro-epithelial complexes would constitute a strong argument against it.

Some of the previously prepared sections were bleached with acetic alcohol or sulphuric bichromate and restained by Masson's trichromic process. This method gives a clearcut differentiation of nerve and connective tissue fibers.

The observations may be described briefly. The interstitial elements between the seminal tubules are essentially identical with those of the albuginea. The cytoplasm does not show any marked difference between endoplasmic and ectoplasmic zones; it contains few vacuoles. For these reasons one may consider them as being in a relatively inactive state. Of course, it is not certain that endo-ectoplasmic differentiation or vacuolization are indispensable aspects of endocrine activity, and the possibility of some degree of hormone production by these interstitial elements is not

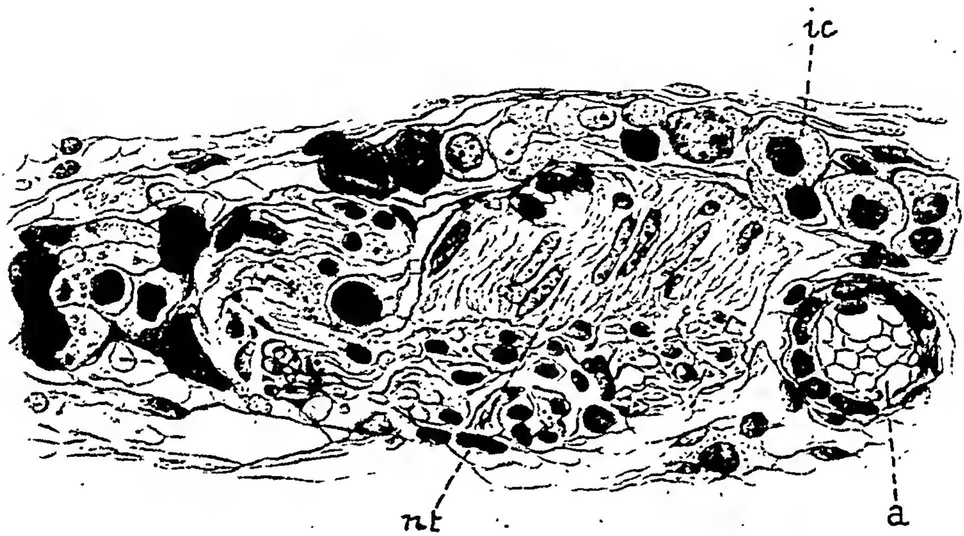

FIG. 2. Detail from another section; same material and technique. Numerous interstitial elements (i.c.) are enveloping a nerve trunk (n.t.) in which a few Schwann cells are seen, *a* artery. Both figures about ×750.

denied. In fact, it is perfectly obvious that before the operation small amounts of androgenic hormones were available, which maintained epididymides of normal male size and of good histologic development.

In the albuginea one finds numerous instances of intermingling or interpenetration of interstitial cells and nerve fibers. Typical areas of restained sections are reproduced in Figures 1 and 2. Both show the intimate interrelationship of interstitial cells and amyelinic nerve fibers, the latter twisting between and around the epitheloid elements. The images are very similar to those presented by hundreds of neuro-epithelial complexes which were observed in normal and cryptorchid males, so that there can be no doubt about the neural nature of these fibers. There is but one difference, and that concerns the aspect of the cytoplasm of the cells of Leydig. In normal and cryptorchid males, many sympathicotropic cells show either a

differentiation of endo- and ectoplasm, a vacuolized aspect of the cyt plasm, or more or less numerous inclusions of crystalloids (of Reink But in the present case, the cytoplasm stains uniformly and appears even density, without special inclusions in the majority of the cells. stated above these histologic features apparently are related to a low secr tory activity of the interstitial cells, and possibly to the low androgen le of this patient.

The reported observations raise the question of the functional impo tance of the innervation of the interstitial cells. Usually one thinks of t nervous and the endocrine systems as of separate control agencies; b instances of interdependence and collaboration are well known. The pre ent case is suggestive of a degree of control of hormone production an release by the autonomous nervous system. Since adrenocortical, inte stitial, and thecal cells are closely related, embryologically and cytolog cally, one may consider qualitative as well as quantitative modificatio Okkels and Sand (7) even hint at the possibility of a separate secretion "still unknown kind" by the Berger cells. The actual value of these su gestions remains to be tested by experimental and physiologic methods.

CONCLUSIONS

The histologic study of the sex glands of a human hermaphrodite (gives evidence of intimate connections between interstitial cells and ner fibers. The appearance of the interstitial cells of the hermaphrodite su gests a relatively low endocrine activity. The possible importance of t innervation of the interstitial cells for the control of volume and quality endocrine production is taken into consideration.

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ADRENAL AND TESTICULAR DEFICIENCY

A COMPARISON BASED ON SIMILARITIES IN ANDROGEN DEFICIENCY, ANDROGEN AND 17-KETOSTEROID EXCRETION, AND ON DIFFERENCES IN THEIR EFFECTS UPON PITUITARY ACTIVITY

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ACCORDING to the theory that there is a second testicular hormone (inhibin), deficiency of this factor, as in the human castrate or in severe testicular failure, is chiefly responsible for the ensuing pituitary hyperactivity. It is generally conceded that androgens arise in the cortex of the adrenal gland as well as in the Leydig cells of the testes. The androgens arising in the testes, together with the androgens and other related substances arising in the cortex of the adrenal, are largely represented in the urine as a group of substances known as 17-ketosteroids. Measurement of these is frequently used to estimate certain hormonal activities of the testes and the adrenal glands. Androgenic activity *per se* is measurable biologically.

In women with ovarian deficiency the cause of pituitary hyperactivity apparently is the loss of estradiol. The high levels of urinary follicle-stimulating hormone (F.S.H.) occurring after castration or the menopause can be reduced by small amounts of estradiol or other estrogens. In Addison's disease in women the loss of androgens from the circulation is apparently the cause of the marked loss of body hair and is indicated also by the extremely low levels of 17-ketosteroids in the urine. It has not been shown that this loss of androgens is followed by increased activity of the anterior pituitary gland, as judged by an increase of urinary F.S.H. The fact that there tends to be a disappearance of basophil (sex hormone-producing (1)) cells from the anterior lobe under such circumstances would lead to the suspicion that the opposite might be the case.

The fact that pituitary hyperactivity occurs in the male as a result of castration or of severe primary testicular failure seems to be acceptable. The occurrence of such high levels of urinary F.S.H., in castrate or eunuchoid men, as shown later, is evidence of this point. Some workers have

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ascribed pituitary hyperfunction resulting from testicular deficiency solely to the deficiency of androgens which exists. Evidence which has accumulated in the past, as well as data presented here, strengthen the opinion that this is not a fact, and that a deficiency of some other hormonal activity is partly and probably chiefly responsible.

A few of the arguments regarding a testicular hormone arising in the germinal epithelium may be mentioned. The idea of an independent endocrine function of the germinal epithelium of the testis was first advanced by Mottram and Cramer (2) in 1923. They showed that application of radium to the testes of the rat produced little effect upon the interstitial cells but rather marked atrophy of the gametogenic elements. Under these circumstances there occurred histologic changes in the anterior lobe of the hypophysis similar to those found after castration. Because the secondary sex characteristics are not obviously affected by destruction of the germinal epithelium, the production of two hormones was suggested by Martins and Rocha (3).

McCullagh (4) and McCullagh and Walsh (5) found that aqueous extracts of the testis caused disappearance of castration changes in the hypophysis, although they brought about no apparent androgen-evoked change in the accessory sex glands. McCullagh and Walsh (5) showed that this effect remained after the lipoid-soluble substances had been removed from such testicular extracts. Roy McCullagh (4) named this water-soluble, pituitary-inhibiting testicular substance "inhibin."

Nelson's (6) studies of cryptorchid animals led him to the belief that castration changes in the pituitary in the presence of damage to the germinal epithelium, are the result of a great sensitivity on the part of the hypophysis and that castration effects of a mild degree did occur in the accessory organs. He felt that such changes need not necessarily be explained on the basis of a second hormone.

Since many workers have shown that sufficiently large doses of androsterone and testosterone, as well as estrogens, will prevent or reverse castration changes, Moore (7) and others have considered the existence of a second testicular hormone improbable.

Törnblom (8) in Uppsala has made extended and well controlled experiments along these lines. His work supports the idea that a second testicular hormone exists. Among other arguments he shows that "giving testosterone to young castrate male rats in such quantity that the weight of the prostate and seminal vesicles somewhat exceeds the weight of these organs in normal animals will not prevent the increase in weight set up in the hypophysis after castration." Such results can readily be duplicated, as shown by the following data from our own laboratory:

	No.	Body Weight	Pituitary	Prostate	Seminal Vesicles
		Gm.	mg.	mg.	mg.
Noncastrate controls	1	255 approx.	6.4	257.0	461.2
	2	258 approx.	6.4	289.2	482.8
	3	260 approx.	5.8	164.8	242.4
	4	262	6.8	282.2	338.6
	5	255	6.2	252.0	392.0
	6	257	7.8	311.6	410.0
Castrated controls	1	290	12.0	atrophied	78.8
	2	280	11.6	atrophied	100.0
	3	287	10.8	atrophied	90.0
	4	255	12.0	none	100.2
	5	278	10.2	atrophied	134.6
	6	272	11.0	atrophied	102.0
Castrate-testosterone injected, 100 micrograms daily	1	318	11.2	433.8	652.4
	2	277	11.4	351.9	509.2
	3	292	8.2	496.6	511.6
	4	305	9.2	321.9	515.6
	5	294	10.4	361.2	486.6
	6	282	10.2	310.0	490.0
Castrate-estrololactone injected, 100 micrograms daily	1	300	14.8	none	131.9
	2	273	13.2	19.0	96.6
	3	276	12.8	atrophied	107.4
	4	300	10.0	29.0	94.8
	5	256	10.0	atrophied	108.0
	6	295	12.0	atrophied	124.0

Törnblom extracted a fraction from bull testes which inhibits the weight increase in the hypophysis of castrated male rats. He calls attention to the fact that this fraction has biologic activities which tally closely with estradiol.

In man two factors may lead to the production of high titres of urinary gonadotropins: 1) castration or severe total testicular damage; 2) oligospermia, in some instances of which there is no clinical evidence of androgen deficiency and in which normal 17-ketosteroid excretion exists. In the latter instance the high gonadotropin excretion cannot be explained on a basis of Leydig cell failure alone and is probably the result of inhibin deficiency. Examples of this are seen in men with cryptorchidism who are large, strong, and normally aggressive, with a normal voice, beard, body

hair, external genitalia and prostate. In other words, these men have not the slightest suggestion of androgen deficiency. Assays for urinary 17-ketosteroids are normal. They have inactive tubular tissue and high titres of urinary F.S.H. There are also such patients as those described by Klinefelter et al. (9) who have oligospermia and gynecomastia, normal urinary 17-ketosteroids, and high urinary F.S.H. These men probably are also examples of deficiency of a second testicular hormone resulting in pituitary hyperfunction, though it is not to be overlooked that these patients merge in clinical appearance with those showing mild eunuchoidism and their appearance does not differ materially from the hypogonadal group with gynecomastia described by Nelson and Heller (10). Data of our own soon to be published indicate that in the presence of testicular deficiency, large doses of androgens fail to reduce the high levels of urinary gonadotropins. It appears also that a defect in gametogenic elements is more intimately connected with such a change than a defect in Sertoli's cells, since similar high titres of gonadotropins may be found in patients whose tubules contain only Sertoli's cells.

It occurred to us that if it were true that diminution of androgens in the body were alone responsible for the pituitary hyperactivity, and if it were also true that androgens in both men and women arise in the adrenal gland, then it should follow that high titres of urinary gonadotropins should be present in patients with destroyed adrenal glands, as in Addison's disease. This should be especially true in women since in them, the only source of androgens is impaired or lost.

In using this type of comparison of testicular and adrenal disease two questions might be raised. First, in testicular failure are the clinical evidences of androgen deficiency, the lowered excretion of androgens and 17-ketosteroids, due to deficiency in testicular hormone directly? From the large amount of experimental and clinical evidence available it appears that in primary testicular deficiency this is true. Certainly there is no evidence to suggest that pituitary or adrenal failure exists to explain such changes. In male rats castration leads to pronounced hypertrophy of both the adrenal cortex and the pituitary (11, 12). Clinically the higher urinary gonadotropins strongly suggest a high degree of pituitary function. In those patients in whom pituitary failure is associated with a pituitary tumor or other local pituitary lesion there are, besides the hypogonadism, other evidences of disease, including signs of failure of thyroid and adrenal functions. This makes it possible to separate these patients from those with primary testicular failure. The second question is: Does the adrenal failure in Addison's disease, with the loss of adrenal hormones, result directly in the diminished androgen effects and excretion and the diminished 17-ketosteroid excretion, or are these the result of associated phenomena? It

might be asked whether such changes are due to pituitary deficiency, and associated testicular failure in men, or to the general debility present.

Pituitary deficiency might be considered as a possible cause of Addison's disease in instances in which adrenal atrophy and not infection is present. If this were true, part of the changes under consideration might be due to testicular deficiency existing as part of Addison's disease. This explanation is not valid because: (a) patients with Simmonds' disease or hypopituitarism with pituitary tumor, though they have adrenal hypofunction, are clearly separable clinically from those with Addison's disease; (b) urinary 17-ketosteroids in patients with hypopituitarism are at a range approaching zero and are much lower than in men with Addison's disease (13); (c) relatively normal sperm counts which exist in Addison's disease indicate active pituitary function; (d) the high gonadotropin titre present in postmenopausal women with Addison's disease is good evidence of strong pituitary function.

The facts strongly favor the idea that the prostatic changes and the lowered androgen and steroid excretion seen in Addison's disease are directly due to a loss of adrenal hormones rather than to testicular deficiency. The prostatic atrophy frequently present in Addison's disease is evidently due to androgen deficiency. Such androgen deficiency is probably not due to testicular hypofunction for the following reasons: (a) testicular atrophy is not usually present in Addison's disease; (b) there are no symptoms of testicular deficiency; (c) sperm counts, though low in some patients, may be entirely normal in others; (d) we have been unable to demonstrate high urinary F.S.H. in Addison's disease except where gonadal failure exists, as in postmenopausal women, or in cases of complete aspermia (case 7) or severe oligospermia.

Low levels of 17-ketosteroid excretion have been observed in Addison's disease when the general health was good due to long continued treatment. This seems to indicate that the low excretion of these materials cannot be explained on the basis of debility.

Androgenic substances and 17-ketosteroids have been shown to be present in the adrenal cortex (14, 15, 16, 17, 18). It is well known that a definite increase in masculinizing materials may arise here in pubertas praecox in boys and in certain adrenal cortical tumors. Loss of these substances from the adrenal itself in Addison's disease seems clearly indicated by the extremely low levels of urinary 17-ketosteroids seen in women with this condition.

METHODS

17-Ketosteroid determinations. Twenty-four hour specimens of urine were used, with the exception of some pooled forty-eight hour specimens. The urine was acidified by the addition of 5 cc. of concentrated sulfuric acid per

100 cc. of urine and immediately hydrolyzed by boiling for ten minutes. The hydrolyzed urine was then extracted in three half-hour periods with redistilled butyl ether, 200 cc. per liter of urine being used for each extraction. The combined butyl ether extracts were washed several times with 1.5 N sodium hydroxide solution, followed by several washings with tap water until the ether layer was clear. In cases in which capon assay was done, half the extract was reserved for that purpose.

Capon assays were done on crude neutral fractions according to methods routinely used in this laboratory (19, 20), five test birds and five controls being used for each test. The butyl ether was removed by vacuum distillation and the residue dissolved in ethyl ether and finally taken up in sesame oil for injection.

The other half was divided into two fractions for 17-ketosteroid determination, earlier tests being made by the Girard (21) method and later ones by adsorption on magnesium oxide (22). Colorimetric determinations were made by the Holtorff-Koch modification (23) of the Zimmermann reaction (24, 25).

The gonadotropic hormone assays were done by one of two methods. In the rat uterine-weight method used in the earlier work (26) the entire twenty-four hour specimen is precipitated with four volumes of 95 per cent alcohol and fractionated according to the method of McCullagh and Bowman (27). The results are expressed in terms of actual rat uterine weight in milligrams.

The mouse uterine-weight method for gonadotropic hormone assays was that of Klinefelter, Albright, and Griswold (28). Immature female mice are used and the gonadotropin to be tested is precipitated from the urine by alcohol. One unit is considered the amount of the hormone which, when injected in five doses over a three-day period in a dilution of 0.5 cc. per dose, causes a minimal uterine weight increase. The uterus is considered enlarged if it weighs more than 7 mg.

RESULTS

Urinary 17-ketosteroids. Urinary 17-ketosteroids in a group of 40 normal men ranged from 4.9 to 18.4 mg. (mostly from 6 to 14 mg.) per twenty-four hours, and in 18 normal women, from 1.5 to 9.7 mg. per twenty-four hours (Table 1).

The range of results of individual tests set forth in Table 2 shows that urinary 17-ketosteroid levels in 13 men with Addison's disease, 5 castrate men, and 6 eunuchoid men, were lower than the normal range established in this laboratory, except in one assay in a man with Addison's disease (*case 1*). It should be noted that the results of the Girard method were usually somewhat higher than those obtained by the magnesium oxide

adsorption method. Results of individual tests in 5 women with Addison's disease (Table 10) were lower than those in men with Addison's disease; this fact is consistent with the generally held idea that since the only source of 17-ketosteroids in women is the adrenal cortex, women with Addison's disease excrete practically no 17-ketosteroids.

Urinary androgens. Levels of androgen excretion in normal individuals are given in Table 3. The results of 41 assays in 20 men are shown. Androgen assays (Table 4) in castrate, eunuchoid, and Addison's disease groups were

TABLE 1. URINARY 17-KETOSTEROIDS* IN NORMAL MEN AND WOMEN 20 TO 40 YEARS OF AGE

Men 40 Patients		Women 18 Patients	
MgO Adsorption		MgO Adsorption	
mg./24 hr.	No. of Cases	mg./24 hr.	No. of Cases
6.0†	4	1.0†	3
7.0	7	2.0	0
8.0	6	3.0	7
9.0	6	4.0	1
10.0	4	5.0	1
11.0	5	6.0	3
12.0	1	7.0	0
13.0	2	8.0	2
14.0	2	9.0	1
15.0	0		
16.0	1		
17.0	1		
18.0	1		

* All 17-ketosteroid assays in this and the following tables represent pure ketonic fractions.

† Each figure represents assays at that level and to 0.9 mg. above it.

abnormally low, with one exception (*case 29*); the levels in some of the tests in this patient fell within the low limits of normal. It is interesting that this patient was probably the most severely eunuchoid in the group. The majority of the assays in patients with Addison's disease were about half of the low limits of normal. In general, these figures are similar to those reported by Callow (29) who found levels of 7, 18, 23, 26, 9, 39, 13, 1, 5 and 20 international units in assays done on 7 patients with Addison's disease. One assay in a patient with Addison's disease after a two-year period of treatment (*case 5*) fell well within normal range, the figures com-

TABLE 2. URINARY 17-KETOSTEROIDS IN MEN WITH TESTICULAR FAILURE AND MEN WITH ADDISON'S DISEASE

Castrate		Eunuchoid		Addison's Disease		
Case	mg./24 hr.	Case	mg./24 hr.	Case	Method: MgO	Method: Girard
					mg./24 hr.	mg./24 hr.
21	6.0	27	8.0	1	3.4	9.0
22	2.0		5.0	2		4.0
	2.0	28	6.0	4		4.0
	1.1*	29	5.0	5		4.0TNF†
	1.0*	30	6.0	6	2.8	5.0
	0.8*	31	8.0TNF†	7	2.5	6.3
	1.3*	32	2.0	8	1.7	
23	5.0			9	2.0	1.0
	4.0*			10		3.0
25	4.0			11		2.0
	3.0*			12		5.0
26	1.0			13	4.0	

* Fractionated by MgO adsorption; others by Girard separation.
† TNF = Total Neutral Fraction.

paring closely with those reported by Koch (30). In neither the castrate nor the Addison's disease group were the 17-ketosteroid levels and andro-

TABLE 3. ANDROGEN ASSAYS IN NORMAL MEN
AGE 24 TO 40 YEARS
(41 assays in 20 men)

Case	I.U.*/24 hr.	No. of Assays	Case	I.U./24 hr.	No. of Assays
1	17	1	11	35	1
2	18	2	12	37	1
3	20	1	13	43	3
4	24	1	14	44	1
5	25	1	15	44	3
6	26	1	16	47	3
7	28	1	17	53	1
8	30	1	18	59	13
9	33	1	19	69	2
10	33	2	20	104	1

* International units.

TABLE 4. URINARY ANDROGENS IN MEN WITH TESTICULAR FAILURE AND MEN WITH ADDISON'S DISEASE, MEASURED BY CAPON COMB GROWTH*

Castrate			Eunuchoidism			Addison's Disease		
Case	I.U./24 hr.	No. of Assays	Case	I.U./24 hr.	No. of Assays	Case	I.U./24 hr.	No. of Assays
21	9	2	29	16.2	5	3	10	1
22	6	2	31	4.4	5	4	10	1
24	12.5	2	32	8.4	5	5	10, 45†	2
26	4	1				6	10	1
27	0	4				9	3	1
						11	7	1
						12	19	1

* Normal range: 18 to 100 I.U./24 hr.
† After two years' treatment with desoxycorticosterone.

gen assays decreased to the same degree. The clinical degree of androgenic deficiency should be clearly distinguished from that indicated by urinary assay.

Prostatic atrophy. The *in vivo* androgenic effects were judged by careful clinical estimation of prostatic size (Table 5). There was consistent and

TABLE 5. DEGREE* OF PROSTATIC ATROPHY IN ADRENAL CORTICAL AND TESTICULAR FAILURE

Castrate		Eunuchoid		Addison's Disease	
Case	Degree	Case	Degree	Case	Degree
21	4+	28	3+	1	0–1+
22	2+	29	4+	2	0
23	4+	30	4+	3	0
24	4+	31	2+	4	3+
25	1+	32	4+	5	1+
26	4+	33	4+	6	0
27	4+			7	3+
				8	2+
				9	0
				10	0
				11	0†
				12	0†
				13	2+

* 0 = normal size; 1+ to 4+ = mild to extreme atrophy.
† Not examined personally.

usually pronounced prostatic atrophy after castration and in eunuchoidism, whereas in men with Addison's disease prostatic atrophy was present only in some cases. In spite of the fact that urinary 17-ketosteroids and androgen levels were low, androgenic effects as judged by prostatic size approximated the normal much more closely in adrenal failure than in testicular failure. However, a tendency to prostatic atrophy in Addison's disease suggests that androgenic effects are decreased in this condition in

TABLE 6. URINARY GONADOTROPIN TITRES IN NORMAL MEN BETWEEN 20 AND 40 YEARS OF AGE

Mouse Units/24 Hours 22 Patients*		Rat Uterine Weight (mg.) 15 Patients	
Mouse Units/24 hrs.	No. of Cases	Uterine Weight (mg.)	No. of Cases
23–26	0	14–20	3
26–53	12	20–40	0
53–105	10	40–60	0
		60–80	7
		80–100	3
		100–120	1

* Ten of the men who had mouse assays had children less than 4½ years of age. All were examined and showed no clinical evidence of endocrine disease.

spite of the presence of intact testes. It should be pointed out that some prostatic atrophy may occur in other chronic debilitating diseases in which adrenal failure may or may not be a factor. The point to which we wish to call attention especially in this respect is that the diminution in androgens in Addison's disease is not connected with high urinary gonadotropins if gonadal function is good. *Cases 6, 8,* and *10* demonstrate this.

Urinary gonadotropins. Tables 6 and 7 show levels of urinary gonadotropins in normal men and women, according to results of the mouse uterine-weight method. In Table 8 it will be seen that in instances of testicular failure, tests by the rat uterine-weight method show high levels, though this method does not show whether or not they are excessive. They approach the maximum response, but all are exceeded by some of the normals shown. Tests by the mouse uterine-weight method all show levels in excess of the upper limit of normal (105 mg. per twenty-four hours). Table 9 shows high levels of urinary gonadotropins associated with severe oligospermia in men who had no clinical signs of testicular deficiency and had levels of urinary 17-ketosteroids within the normal range (an exception is

seen in one of two assays in *case 33*). This we interpret as due to lack of "inhibin."

Table 8 shows the results of gonadotropin assays in 7 of the 15 men with Addison's disease. The gonadotropins were normal or low in all instances but one (*case 7*), in which there was absence of one testis and oligospermia. This case shows that a high level of gonadotropins may be present in Addison's disease when testis damage also exists. On the other hand, *cases 6* and *9* indicate that the low levels of 17-ketosteroids and low androgens in Addison's disease were not sufficient in themselves to cause an

TABLE 7. URINARY GONADOTROPIN TITRES IN NORMAL WOMEN BETWEEN 19 AND 36 YEARS OF AGE*

Mouse Units/24 hrs. 20 Patients	Age (Yrs.)	Day of Cycle	Length of Cycle, Days
13– 26*	20	14	26
13– 26	21	13	32
13– 26	21	28	29
13– 26	22	23	29
13– 26	22	27	28
13– 26	23	8	24
13– 26*	25	10	?
13– 26	35	18	28
26– 53	30	24	26
26– 53	36	26	27
26– 53	?	16	?
53–105	19	16	28
53–105	20	17	32
53–105	20	20	47
53–105	22	14	?
53–105	25	18	28
53–105	28	18	34
53–105	30	12	28
53–105	?	10	?
53–105*	?	17	?

SUMMARY

Range in M.U.	Patients
13– 26	8
26– 53	3
53–105	9
	20

* All had young children or were shown to have normal vaginal smears except those marked *, in whom smears indicated a mild ovarian deficiency.

TABLE 8. URINARY GONADOTROPINS IN MEN WITH TESTICULAR FAILURE AND MEN WITH ADDISON'S DISEASE

Castrate			Eunuchoid			Addison's Disease			
Case	Rat Uterine Weight* (mg.)	Mouse Units	Case	Rat Uterine Weight* (mg.)	Mouse Units	Case	Rat Uterine Weight* (mg.)	Mouse Units	Sperm Counts (total)
21	91	106–212	28		105+	1		26–53	2 per HPF
22	94	212–424	29		192+	6	20		1165 million
24	89		30		192+	7	80	<105	0
27	High†		31		192–288	8		>6.6	38 million
			32	20					71 million
			33		318+	9		6–13 on 4/12/44	
								13–26 on 6/29/44	
								26–53 on 6/30/44	impotent
						10		>6.6	436 million
						13		6.6–13	

* Rat uterine weight in normal uninjected animals weighing between 30 and 40 Gm. = 17 to 21 mg.

† Friedman test—corpora lutea 3+.

TABLE 9. URINARY GONADOTROPINS AND 17-KETOSTEROIDS IN MEN WITH OLIGOSPERMIA

Case	Rat Assay Uterine Weight (mg.)	Mouse Units	17-Ketosteroids mg./24 hrs.	Sperm Count (HPF)
34	81	480–633	8	occasional
35	82, 74	288–384	11, 13, 10	occasional
36	64	105–212	11, 7	few
37	89, 79	105–212	10	1
38	78	318–424	14	1
39	89, 79, 75	105–212	10	1

elevation in gonadotropins, in definite contradistinction to the effect produced in castration.

Table 10 shows that urinary gonadotropins in women are not elevated in the presence of very low levels of 17-ketosteroids alone (*case 16*) but that when ovarian failure is superimposed, high levels of gonadotropins are produced.

COMMENT

It is interesting that in *case 1* and *case 8* of Addison's disease, prostatic atrophy was present, indicating a lowered androgenic effect with no in-

crease in gonadotropin excretion. In eunuchoidism, if testicular failure is severe enough to produce prostatic atrophy, it would be expected to be severe enough to produce a distinct rise in the excretion of gonadotropins. It is interesting also that in Addison's disease, when mild prostatic atrophy exists in the presence of normal sperm production, no increase in gonadotropins has been found (*case 6*).

In our patients with Addison's disease without evidence of gonadal failure, no increase in urinary gonadotropins existed in spite of the low 17-ketosteroid levels.

TABLE 10. URINARY GONADOTROPINS AND 17-KETOSTEROIDS IN WOMEN WITH ADDISON'S DISEASE

Case	17-Ketosteroids mg./24 hrs.	Gonadotropins (M.U.)	Remarks
14	1	105–212	Age 49. Menopause. Amenorrhea 5 years. Had no hot flashes.
15	1.0	105–212 {6–13 in hospital 105–212	Age 35. Ovarian deficiency. Amenorrhea 11 months. Endometrium too scant for biopsy. Vaginal smear deficient.
16	2.0	6–13	Age 33. Menses irregular. Vaginal smear 3+. Premenstrual endometrium showed full progestational response.
17	1.1	318–424	Age 49. Menopause. Last menses two years ago. Hot flashes.
18	1.1	105–212	Age 57. Menopause for 2 years. Vaginal smear similar to that of a castrate.
19	1.5	13–26 13–26	Age 42. Menses regular. Vaginal smear 3+ to 4+.
20	2.1	105–212	Menopause. Hot flashes about age of 35.

SUMMARY

1. Levels of urinary androgens and 17-ketosteroids found in normal subjects are compared with low levels found in testicular failure and in Addison's disease in men. In the men reported in this paper, urinary 17-ketosteroid levels in Addison's disease and in severe testicular deficiency, approximate half of normal.

2. Judged by prostatic size, androgen deficiency is greater in testicular failure than in Addison's disease.

3. Titres of urinary gonadotropins in normal subjects are compared with those found in testicular failure and oligospermia and in men and women with Addison's disease.

4. Low urinary 17-ketosteroid levels and androgen levels in men with Addison's disease are not associated with high titres of urinary gonadotropins.

5. Severe oligospermia without signs of androgen deficiency and with normal 17-ketosteroids is associated with high excretion levels of gonadotropins.

6. Gonadotropin excretion is normal in Addison's disease (despite loss of adrenal androgens) when gonadal function is normal, and is increased when gonadal damage is superimposed.

7. The data presented are interpreted to mean that while androgen deficiency in men and women has little power to cause pituitary hyperactivity, ovarian deficiency and testicular gametogenic deficiency do have such power. This is considered an additional argument in favor of the existence of a second testicular hormone.

PROTOCOL SUMMARIES

Addison's Disease (Men)

Case 1. Age 33.

Diffuse pigmentation, black freckles, and weakness for fifteen months. Weight loss 23 pounds. A typical crisis had occurred previously. B.P. 114/70 during desoxycorticosterone therapy. Testes of normal size and consistency. Prostate smaller than average. Excellent response to treatment for four years. Died later in crisis.

Case 2. Age 21.

Extreme anorexia and weakness associated with diabetes mellitus. Many black freckles for five months. B.P. 100/70. Water excretion test positive. Great improvement during desoxycorticosterone therapy for four years. Testes and prostate normal. Living.

Case 3. Age 57.

Extreme weakness for four months. Increasing pigmentation. Black moles. Weight loss 10 pounds. B.P. 80 systolic. Water excretion test positive. Response to cortical extract good for two years. Testes and prostate normal. Later died with tuberculosis.

Case 4. Age 29.

Examined in typical adrenal crisis. Deep diffuse pigmentation. B.P. 96/54. Blood chloride 423 mg. per cent. X-ray examination showed pulmonary tuberculosis and adrenal calcification. Response to therapy excellent for four and one-half years. Prostate decidedly atrophic. Testes normal. Died following acute infection.

Case 5. Age 18.

Extremely dark diffuse pigmentation for six years. Extreme weakness for several months. B.P. 72/60. Water excretion test positive. Response to treatment excellent for

five years. Prostate smaller than average. Testes normal. Died following one day of fever.

Case 6. Age 34.

Nausea, vomiting, and weakness for two months. Vitiligo. Black freckles. Dark palmar creases. B.P. 92/78. Water excretion test positive. Working regularly for six and one-half years. Prostate soft and boggy. Testes normal.

Case 7. Age 54.

Weakness, salt craving, and hiccough for four months. Generalized pigmentation and black freckles, especially on pressure areas. B.P. 96/68. Water excretion test positive. Orchidectomy for tuberculous orchitis twelve years previously. Prostate decidedly atrophic. Impotence. Aspermia. Hot flashes. Following treatment with pellets of desoxycorticosterone, improvement was excellent for four years. Living.

Case 8. Age 24.

Increasing pigmentation for one year. Excessive fatigue for two months. Black freckles for one month. B.P. 96/50. Daily fever. Pulmonary tuberculosis evident by x-ray examination. Right adrenal calcified. Prostate moderately atrophic. Improvement during therapy for one year. Drinking bout followed by coma, convulsions, death.

Case 9. Age 39.

Increasing diffuse pigmentation of skin and buccal mucosa for six months. Dark freckles, weakness, hiccough and nausea. B.P. 86/62. Water excretion test positive. Testes and prostate normal size. Response to treatment excellent for three years. Autopsy showed tuberculous adrenals.

Case 10. Age 58.

Weakness for four months. Nausea and pigmentation of skin and gums. B.P. 70/50. Spastic contraction of skeletal muscles. Testis and prostate normal size. X-ray examination disclosed pulmonary tuberculosis. Died in crisis.

Case 11. Age 49.

Weakness, nausea, vomiting, and inconstant indigestion for eight years. Pigmentation of face, hands, and lips for six years. Adrenal crisis diagnosed at Johns Hopkins Hospital. Urine culture positive for tuberculosis. (From notes by Dr. George W. Thorn.)

Case 12. Age 48.

Pigmentation and vitiligo for eight years. Weakness and anorexia for seven years. Repeated crises. Improvement followed treatment with desoxycorticosterone pellets. (From notes by Dr. George W. Thorn.)

Case 13. Age 51.

Incapacitating weakness, anorexia, and indigestion for two months. Loss in weight of 15 pounds. Black freckles. B.P. 80 systolic. Water excretion test positive. Prostate slightly atrophic. Response to therapy good.

Addison's Disease (Women)

(For further notes concerning ovarian function see Table 10.)

Case 14. Age 49.

Increasing weakness for one and one-half years. B.P. 80/60. Axillary and pubic hair

very sparse. Wilder test positive. Water excretion test positive. Treatment with desoxycorticosterone pellets, sodium chloride and cortical extract was followed by marked improvement. Amenorrhea for five years. Living.

Case 15. Age 35.

Recurrent attacks of weakness, nausea, and abdominal distress for three years. Diffuse smoky pigmentation of the skin and dark freckles. Axillary and pubic hair very sparse. B.P. 98/68. Water excretion test positive. Treatment with desoxycorticosterone pellets was followed by decided improvement. Associated ovarian failure. Dead.

Case 16. Age 35.

Frequent nausea and vomiting for one year, and diminishing strength. Pigmentation of skin over pressure areas. Typical crisis. B.P. 90/75. Axillary and pubic hair were almost less than normal. Water excretion test positive. Improvement great after desoxycorticosterone pellets and cortical extract. No ovarian failure. Living.

Case 17. Age 49.

Increasing pigmentation of skin for three years. Weakness for two years. Vomiting frequent. Axillary hair absent, pubic hair sparse. B.P. 100/70. Water excretion test positive. Response to cortical extract excellent for two and one-half years. Menopause. Living.

Case 18. Age 57.

Typical pigmentation of skin for two years. Progressive increase in weakness, anorexia, and nausea. B.P. 80/50. Considerable improvement during treatment with cortical extract. Menopause. Living.

Case 19. Age 42.

Weakness, nausea, vomiting, and diarrhea for six weeks. B.P. 72/58. Pigmentation of skin and buccal mucosa. Marked improvement during treatment with desoxycorticosterone implants and aqueous adrenal cortical extract.

Case 20. Age 62.

Increasing pigmentation of skin for two years. Muscular cramps for one year. Pronounced weakness for two months. B.P. 100/70. Excellent response to desoxycorticosterone and salt. Menopause. Living.

Castrate Men

Case 21. Age 66.

History of bilateral orchiectomy for "testicular pain" twelve years ago. Psychoneurosis severe. Physical condition good.

Case 22. Age 52.

Left orchiectomy for trauma six years ago. Right orchiectomy for orchitis. Hot flashes, nervous tension, and impotence controlled by testosterone propionate 25 mg. intramuscularly three times weekly.

Case 23. Age 51.

Bilateral orchiectomy four years previously for carcinoma of prostate. No hot flashes. No evident metastases one year after time of assays.

Case 24. Age 46.

Bilateral orchiectomy for tuberculous orchitis twelve years before assays. Ass
tuberculosis of urinary tract recognized one year before assays. Nutrition good. N
ness and hot flashes present.

Case 25. Age 69.

Bilateral orchiectomy. Assays one year later during apparent good general
Impotence and hot flashes present.

Case 26. Age 47.

Bilateral orchiectomy for tuberculous orchitis twelve years before assays.
and impotence present but general health good. No hot flashes.

Case 27. Age 64.

History of bilateral orchiectomy for suspected testicular tumor. Nervous
extreme. Frequent hot flashes. Symptoms relieved by testosterone propionate.

Eunuchoid Men

Case 28. Age 41.

Mumps at age 7. Typical eunuchoidism. General appearance and sexual m
approximately normal for age 15. Testes decidedly atrophic. Sella turcica nor
x-ray examination. Aspermia.

Case 29. Age 19.

No history of orchitis. General and sexual maturity arrested at approximat
12-year level. Testis biopsy showed tubular hypoplasia, aspermia, numerous S
cells, rare interstitial cells.

Case 30. Age 37.

Typical severe eunuchoidism. Genitalia of prepuberal proportions. Prostate at
Aspermia.

Case 31. Age 18.

Appearance of moderate immaturity. Penis, testes, and prostate hypoplastic.
mia.

Case 32. Age 36.

General skeletal and sexual maturity arrested. Testes partially cryptorchid an
tate aplastic. Aspermia. Sella turcica normal by x-ray examination.

Case 33. Age 32.

Eunuchoidism from testicular atrophy following orchidopexy at the age of 12.
tate aplastic. Aspermia.

Aspermia and Severe Oligospermia

Case 34. Age 21.

Large bilateral hydrocele. Testes palpable and atrophic. Semen volume 8.1 cc
spermatozoon. Signs suggestive of mild androgen deficiency.

Case 35. Age 28.

Mild eunuchoidism, cryptorchidism, and hypospadias. Prostate half normal size. Rare inactive spermatozoon in semen. Sella turcica normal by x-ray examination.

Case 36. Age 27.

Appearance slightly immature. Left gynecomastia. Penis and prostate normal size. Testes half normal size. Semen volume 2.7 cc. One spermatozoon found. Sella turcica normal by x-ray examination.

Case 37. Age 36.

Genital development and secondary sex characteristics normal, except right testis slightly small. Severe oligospermia. Sella turcica normal by x-ray examination.

Case 38. Age 34.

Mumps at age 10. No recognized orchitis. Genitalia and secondary sex characteristics normal, except left testis small, and prostate one-third normal size. Semen volume 1 cc. Azoospermia.

Case 39. Age 27.

General appearance suggests mild androgen deficiency. Bilateral gynecomastia. Bilateral atrophy of testes. Aspermia on repeated examination. Testis biopsy showed no spermatogenic activity. Interstitial cells appeared normal.

Wassermann and Kahn tests were negative in all patients except in *case 24*, where there was no evidence of active syphilis. In the men with eunuchoidism and aspermia or oligospermia there was no history of venereal disease.

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ANALOGIES BETWEEN URINARY 17-KETO-STEROIDS AND URINARY "ESTROIDS," AS DETERMINED MICROCHEMICALLY*

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SEVERAL years ago Callow and Callow (1) demonstrated that the microchemical determination of 17-ketosteroids could yield valuable information regarding endocrine function in man and other animals. This finding has since been substantiated in many other laboratories (2–6). Nevertheless, considerable opposition to the procedure was raised originally on the grounds that: (a) the urinary 17-ketosteroids constitute a mixture; (b) their value is considerably higher than a biologically equivalent amount of androsterone; and (c) they vary with changes in both testicular and adrenal function. Similar objections can be raised with respect to urinary components related to estradiol.

Degradation products of estradiol in urine

Like testosterone, estradiol is converted within the human organism into degradation products of lower biological potency. The best known of such degradation compounds in the female are estrone and estriol (6). Smith and Smith (7) have also concentrated from human urine a substance which tentatively they regard as a lactone. Furthermore, in animals even after oöphorectomy certain estrogenic effects can be detected, especially in the presence of adrenal hyperplasia (8). Evidence is accumulating that these effects are due to materials which resemble estradiol chemically (9). Under appropriate chemical conditions all of these substances, as isolated from urine, give color reactions similar to estradiol. In the present paper the term "urinary estroids" is used to signify a mixture of steroids which, after extraction from urine and appropriate partial purification, can be estimated microchemically in terms of estradiol equivalents.

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Photometric measurements of estradiol-like compounds

The quantitative study of urinary "estroids" has been delayed for lack of methods sensitive enough to detect the relatively minute amounts present in nonpregnant individuals. In pregnancy, Bachman and Pettit (10) and Stimmel (11) have suggested techniques which are fairly satisfactory for daily excretions of approximately 1 to 3 milligrams daily. Unfortunately, in the absence of pregnancy, the average daily human excretion is less than 100 micrograms even in healthy young women. Friedgood and Garst (12) have applied ultraviolet absorption for this purpose whereas Bates and Cohen (13), Jailer (14) and Finkelstein, Hestrin and Koch (15) have used fluorescence. The data in the present report are based upon a modified Kober's reaction (16). Ordinarily the vermilion shade produced by this phenolsulfonic reagent on reacting with estrone (or estradiol) is obscured by brownish pigments resulting from other urinary chromogens. In the present investigation, therefore, most of these interfering pigments were removed by suitable extraction *after* development of the Kober color.

Simultaneous determination of 17-ketosteroids and of estradiol-like substances

Periodically the problem of androgen:estrogen antagonism appears in the literature (17). The interest in the ratio of these hormones lies in the fact that in certain respects, e.g., in their effect upon the capon's comb, their action is antagonistic or antithetical. The older data were based upon biological assays in which great variability of the test animals combined with great variability among individual subjects to yield widely divergent results. Nevertheless, even under these difficult technical conditions, it was possible to show (18) that an inversion of the ratio of these antithetical substances occurred when men were compared with women. For example, the average woman excreted only half as much mixed androgen as the average man, whereas she excreted at least twice as much mixed estrogen. In the present report the term "antithetical ratio" will be used to signify the arithmetical value derived by dividing the urinary excretion of "estroids" (expressed in micrograms) by the excretion of 17-ketosteroids (expressed in milligrams). This procedure suffers from all the physiologic objections of the older estrogen:androgen ratios *except* that more consistent values are obtained because the variance due to test animals is eliminated.

METHODS

A. For Determination of 17-Ketosteroids. The procedure for 17-ketosteroids was essentially that of Cahen and Salter (4) as applied by

Salter, Cahen and Sappington (19). This method has given results which closely approximate values obtained by Drs. Fuller Albright (20), Lawson Wilkins (21) and Ralph Dorfman (22). *Identical urines* from the patients in their studies were kindly supplied to us for the comparison.

For example:

		mg./24 hrs.		*mg./24 hrs*
Urine #1	Albright	7.8	Salter	7.8
Urine #2	Albright	9.0	Salter	9.1
Urine #3	Dorfman	20.0	Salter	19.9 (Wilkins' patient.)
Urine #4	Wilkins	8.6	Salter	7.5
Urine #5	Wilkins	15.3	Salter	16.6

In any case the procedure gives a consistent range of values in normal healthy young adults. Ordinarily the extract of 17-ketosteroids was made in conjunction with the extraction of estradiol-like substances ("estroids"). Occasionally, however, a small aliquot sample (from 20 to 100 ml.) was extracted separately, as suggested by Drekter, Pearson and McGavack (23). In the latter case however, the colorimetric procedure was that of Cahen and Salter (4) so that all results are strictly comparable.

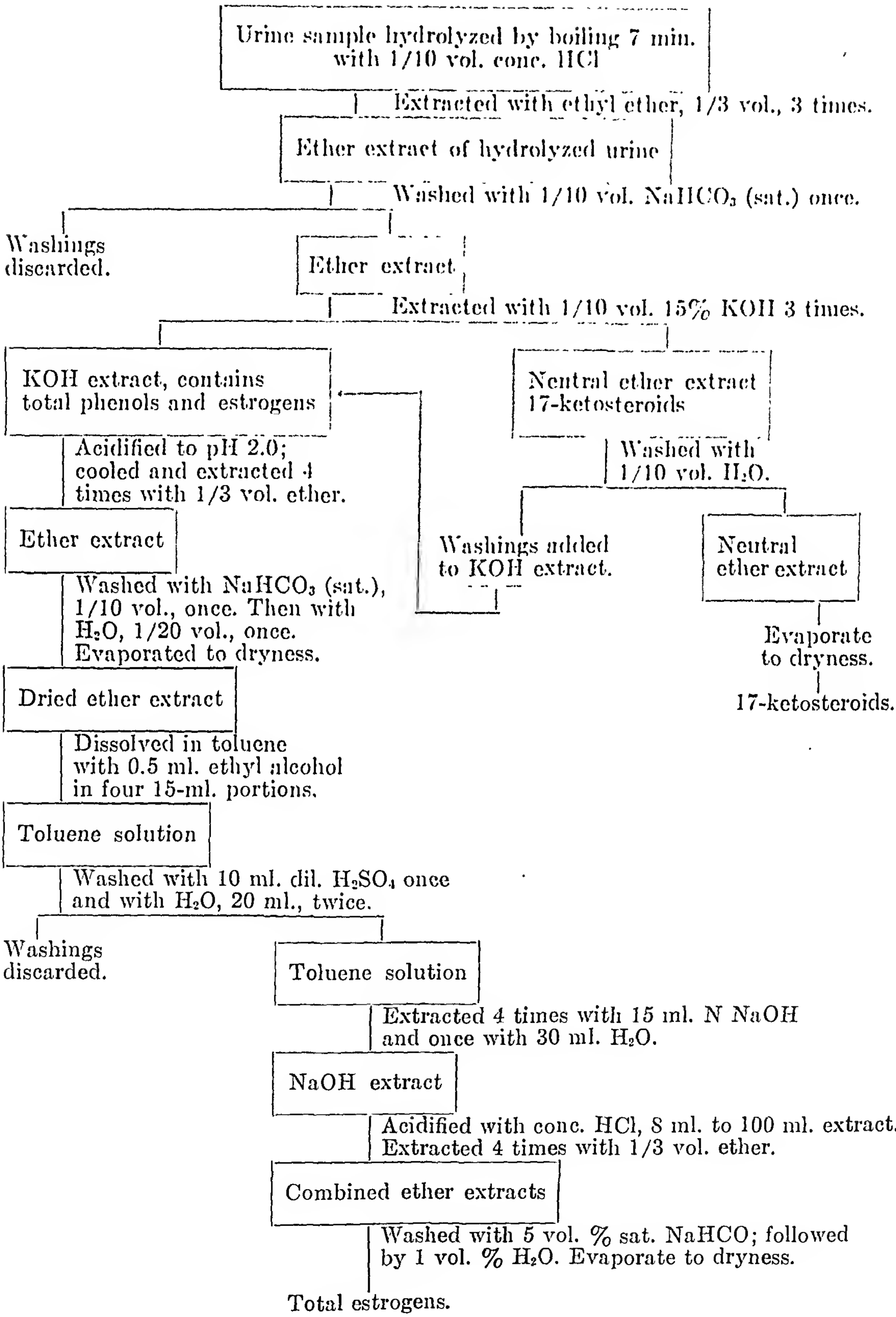
B. Schema for Extracting Urinary "Estroids." The accompanying schema (See next page) indicates the extraction procedure followed. It is a combination of the procedures of Pincus (24) and Bachman and Pettit (10). Usually a six-hour excretion was studied, i.e., one-fourth of a mixed 24-hour specimen.

C. Microcolorimetric Determination of "Estroids" (Estradiol-like Chromogens). The dried "estroids" can be kept in an ice box for many days without perceptible deterioration. It is possible to fractionate the material, according to the method of Bachman and Pettit (10), in an attempt to identify the estrone-estradiol (OD) moiety and the estriol (T) fraction. In our experience, however, this method has not operated successfully for the small amounts under consideration.

For the final step of the analysis, the dried "estroids" are dissolved in pure di-ethyl ether. Each sample is then transferred to a test tube (150×15 mm.) and evaporated to dryness.

Reagents:

1. *Phenolsulfonic acid* (Kober's reagent). Melt the phenol (reagent grade) by placing in a water bath at 60°C. and measure 100 ml. into a previously warmed graduate. Measure 156 ml. of concentrated sulfuric acid into a beaker set in an ice bath, and slowly add the melted phenol, stirring constantly. Add 128 ml. of water slowly while stirring. Heat will be evolved and the reagent will deepen in color unless it is kept cold during the mixing procedure.


Urine sample hydrolyzed by boiling 7 min. with 1/10 vol. conc. HCl
Extracted with ethyl ether, 1/3 vol., 3 times.
Ether extract of hydrolyzed urine
Washed with 1/10 vol. $NaHCO_3$ (sat.) once.
Washings discarded.
Ether extract
Extracted with 1/10 vol. 15% KOH 3 times.
KOH extract, contains total phenols and estrogens
Neutral ether extract 17-ketosteroids
Acidified to pH 2.0; cooled and extracted 4 times with 1/3 vol. ether.
Washed with 1/10 vol. H_2O.
Ether extract
Washings added to KOH extract.
Neutral ether extract
Washed with $NaHCO_3$ (sat.), 1/10 vol., once. Then with H_2O, 1/20 vol., once. Evaporated to dryness.
Evaporate to dryness.
Dried ether extract
17-ketosteroids.
Dissolved in toluene with 0.5 ml. ethyl alcohol in four 15-ml. portions.
Toluene solution
Washed with 10 ml. dil. H_2SO_4 once and with H_2O, 20 ml., twice.
Washings discarded.
Toluene solution
Extracted 4 times with 15 ml. N NaOH and once with 30 ml. H_2O.
NaOH extract
Acidified with conc. HCl, 8 ml. to 100 ml. extract. Extracted 4 times with 1/3 vol. ether.
Combined ether extracts
Washed with 5 vol. % sat. NaHCO; followed by 1 vol. % H_2O. Evaporate to dryness.
Total estrogens.

2. *Dilute sulfuric acid.* Add 3 parts by volume of conc. sulfuric acid (C.P., analytical reagent) to 7 parts of water.
3. *Chloroform-ethyl acetate mixture.* Add 2 parts by volume of chloroform (analytical reagent) to 1 part of ethyl acetate (C.P., absolute).
4. *Chloroform-caprylic alcohol mixture.* Mix 25 parts by volume of chloroform (analytical reagent) with 8 parts of n-capry alcohol (C.P., Fisher Scientific Company).
5. *Petroleum ether* (analytical reagent).

Standards:

Estrone, 1 microgram per ml. in 95 per cent alcohol.
Estriol, 1 microgram per ml. in 95 per cent alcohol.

Color Reactions for Determinations of "Estroids"

The following directions indicate the method of developing the color:

1. Into a convenient number, e.g., 12, of clean and dry test tubes (15×100 mm.) place
   a. The reagent blank
   b. Pure estriol (T) standard
   c. Pure estrone (OD) standard
   d, e, f, etc. Unknown samples
2. Dry the tubes in a vacuum desiccator for one hour before the experiment starts. (Meanwhile heat up a bath containing N-butyl phthalate to 130°C. and start the motor stirrer therein. Prepare an ice bath.)
3. After drying the tubes for an hour in the desiccator, remove them and add Kober's reagent as follows:
   a. e.g., if the aliquot of urine is 25 per cent to 35 per cent of a 24-hour excretion, use 1.5 ml. of Kober's reagent and 6 ml. H_2SO_4. Add only Kober's now, H_2SO_4 later, in step 7.
   b. e.g., if the aliquot of urine is 35 per cent to 50 per cent of a day's excretion, use 3 ml. of Kober's reagent and 12 ml. H_2SO_4.
   c. e.g., if the aliquot is less than in "a.", use 0.75 ml. of Kober's reagent and 3 ml. H_2SO_4.

   Always maintain Kober's reagent in proper proportion to H_2SO_4.
4. Heat the tubes at 130°C. for 20 minutes *exactly*. To this end, place the tubes in the bath in series, one every 15 seconds. Agitate the contents of each tube for ten seconds. Remove the series of tubes from the bath in the same order every 15 seconds and stir each tube for ten seconds thereafter.
5. Leave all tubes of the series in ice at least 5 minutes and take all tubes out of the ice together.
6. Allow all tubes to warm to room temperature.
7. Add H_2SO_4 to each tube as specified in step no. 3 above and stir well.
8. From the large tubes pour approximately 3 ml. of each colored mixture into round bottomed test tubes.
9. To each small tube add 1.5 ml. of the ethyl acetate-chloroform mixture. Shake each tube and remove the top layer (containing a dirty precipitate) with a suction pipette.
10. Add 0.5 ml. of petroleum ether. Shake the contents of each tube and remove the dirty supernatant liquor. Most of the dirt comes off at this stage; therefore, draw off as cleanly as possible.

11. Add 1.5 ml. of the capryl alcohol-chloroform mixture, shake, and draw off the top layer. This reagent should be prepared just before using. It contains 8 ml. capryl alcohol and 25 ml. of chloroform.
12. Add 0.5 ml. of petroleum ether and shake. *This time the supernatant layer is not drawn off.*
13. Let each sample stand for one hour at room temperature. Then remove petroleum ether with a suction pipette and transfer to a small colorimeter tube. At least 2 ml. total volume is required in each tube for colorimetric readings.

Colorimetry:

The method of colorimetry involves a green filter (No. 540) and a blue filter (No. 420) as suggested by Stimmel (11). The readings are conveniently converted into corresponding concentrations by the use of a nomogram, pictured in Figure 1. Each investigator should prepare his own nomogram, using known standards, as reported by Oesterling and Salter (25).

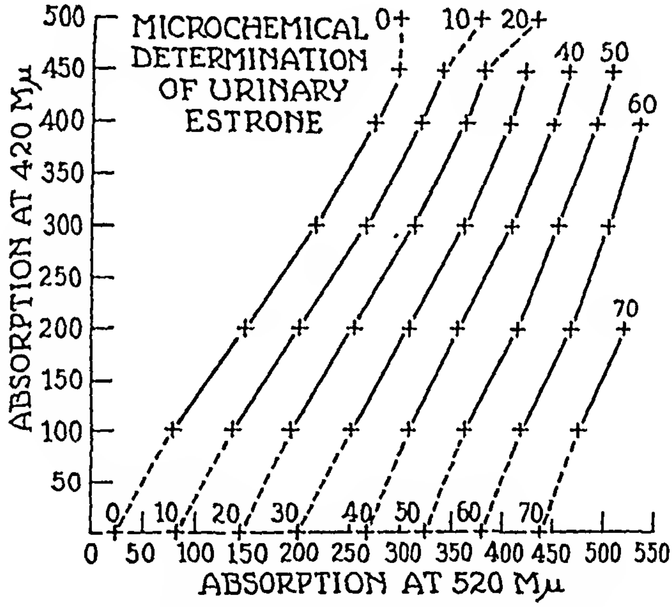


FIG. 1. A nomogram relating photoelectric colorimeter readings to the estrone present.

D. Representative Values for Normal Males. In addition to the normal excretions reported previously from this laboratory (4, 19, 26), in the course of the present investigation data were obtained from normal male students and members of the University staff. These are summarized in Table 1. The average daily 17-ketosteroid excretion from 24 young males was 19.8 ± 3.5 milligrams. The average daily "estroid" excretion from 19 young males was 16.7 ± 9.4 micrograms. The antithetical E/A ratio for 16 young males averaged 0.86 ± 0.49.

E. Representative Values for Normal Females. In the healthy mature female (nonpregnant) the problem of citing normal values is complicated by the dimension of time. The 17-ketosteroid excretion remains remark-

TABLE 1. URINARY DAILY EXCRETION OF 17-KETOSTEROIDS AND "ESTROIDS" IN NORMAL YOUNG ADULT MALES, AS DETERMINED MICROCHEMICALLY

Patient	Age Yrs.	Height	Weight	17-Ketosteroids (hydrolyzed) milligrams	Total "Estroids" (hydrolyzed) micrograms	E/A Ratio
Second Decade		ft. ins.	lbs.			
1. Mr.	19	5 9½	155	13.1	2	0.18*
Third Decade						
1. Hn.	30	5 11½	190	13.4	7	0.49
2. Go.	26	5 11	160	15.7	8	0.51
3. Lw.	22	5 7	135	15.9		
4. Os.	22	5 10½	155	16.2	8	0.48
5. Rg.	21	5 8	145	16.4		
6. Be.	23	5 11	165	17.0		
7. Cm.	21	6 2	155	17.9	23	1.28
8. Br.	22	5 8½	154	18.5	29	1.55
9. Eg.	21	5 9½	165	18.6	26	1.40
10. Sh.	23	5 8	155	18.8	12	0.64
11. Jn.	21	5 9½	140	18.8	17	0.90
12. Bs.	22	6 2½	190	18.9	18	0.96
13. Bt.	25	5 8½	151	18.9	37	1.95
14. Tt.	26	5 8½	150	19.1, 19.1 } 19.1	6	0.31
15. Bn.	23	6 3	180	19.9		
16. Rl.	22	6	165	20.0		
17. Rn.	20	5 10	155	20.2		
18. Js.	20	5 11	150	22.0	11	0.48
19. Sn.	22	5 9	133	23.1, 21.4 } 22.3	27	1.18
20. Nm.	21	5 10	155	22.8	18	0.81
21. Le.	26	5 10	157	25.0	8	0.31
22. Gr.	22	6 1	170	25.1		
23. Pr.	21	6	148	26.2	12	0.47
24. Ce.	23	5 11¾	150	27.1		
				Av. 19.8 ±3.5	Av. 16.7 ±9.4	Av. 0.86 ±0.49
Fourth Decade						
1. Tr.	35	5 7	150	13.8	12	0.88
2. McC.	31	6 3½	190	16.6	17	1.00

* "In the calculation of E/A ratios presented in this report, the arithmetic has been performed *before* the last significant figure of each experimental value has been rounded off. This procedure, which may give the impression of faulty arithmetic, is preferable because it avoids incorporating into the results the slight error of smoothing the last significant figure."

ably constant throughout the menstrual cycle, but the "estroid" excretion varies with ovarian follicular activity. In Figure 2 are presented the findings for both 17-ketosteroids and "estroids" in a normal woman 26 years old.

COMPARATIVE RESULTS

Because the microchemical determination of estrogen-like substances in the urine is now becoming feasible, certain analogies between these

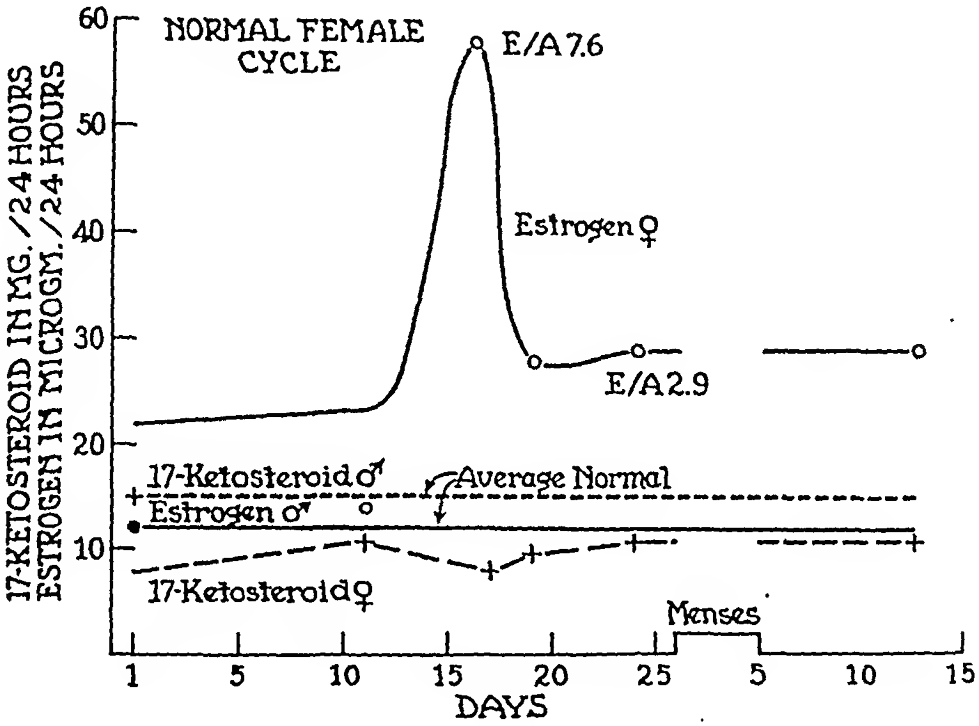


FIG. 2. Variation in urinary "estroids," as determined microchemically, throughout the menstrual cycle of a normal woman, aged 26.

"estroids" and the 17-ketosteroids should be noted at this time, in the hope that useless controversy and futile experimentation may be avoided. It is the purpose of this report to point out five analogies between the respective microchemical estimations of 17-ketosteroids and "estroids."

I. **The effect of age.** It is now clearly established that even in reasonable health, the 17-ketosteroid excretion of mature men declines with advancing years. Representative reports on this point are those of Moore (27) and Hamilton (28). In Figure 3 are illustrated data from the author's laboratory combined with those of Hamilton. In contrast to this trend, the urinary "estroids" tend to increase slightly with age. This second trend is illustrated in Figure 4, in which simultaneous determinations of urinary 17-ketosteroids and "estroids" are pictured for several age groups.

Consequently there is a numerical increase in the antithetical E/A ratio in later life. It might be argued, of course, that this altered ratio is an indication of endocrinological degeneration and therefore is pathological. It seems more practical, however, to establish "normal" standard values for each age group.

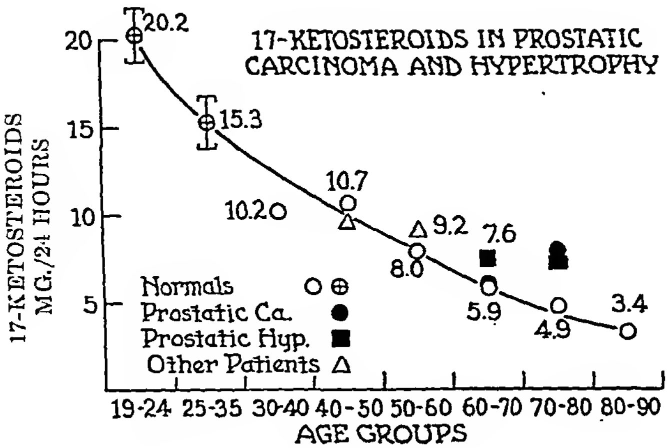


FIG. 3. Effect of age on the daily urinary excretion of 17-ketosteroids in men.

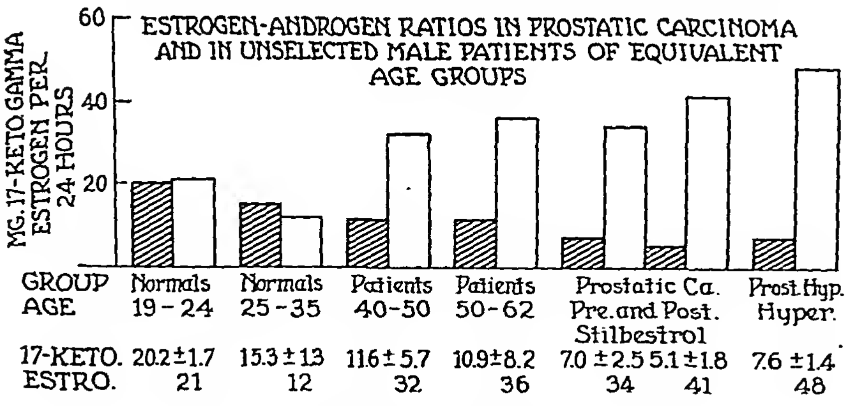


FIG. 4. Parallel determinations of urinary 17-ketosteroids and "estroids" for several age groups.

Women in the reproductive age groups present the further complication of the cyclic swing in "estroid" excretion, already illustrated in Figure 2. Near the menstrual period some normal women present absolute values both for 17-ketosteroids and for "estroids" rather similar to the extremes of normal men. Even at this time, however, the antithetical E/A ratio usually remains higher in the female than the arbitrary "upper limit of normality" for males of the same age. A further complication which re-

quires study is the origin of "estroids" found in the castrated or post-menopausal female—possibly the result of adrenocortical activity.

II. **The influence of medication with sex hormones.** Of special interest is the effect of stilbestrol and its derivatives on the urinary "estroids." It has been demonstrated repeatedly (29–31) that large doses of stilbestrol will result in a perceptible urinary excretion of this drug or its esters. Accordingly, after administration of stilbestrol, bioassay of the urine will demonstrate increased estrogenic activity. Pure stilbestrol, however, does not react appreciably with the Kober reagent (16), so that its presence in urine is not recorded microchemically unless a specific analytical procedure (32) is introduced for this purpose. Figure 5 indicates that the daily administration of stilbestrol in doses of from 5 to 20 mg. had no significant effect upon the so-called endogenous "estroid" excretion. Nevertheless, the administration of this medication in large doses may alter the antithetical ratio by lowering 17-ketosteroid excretion, as shown in Figure 5.

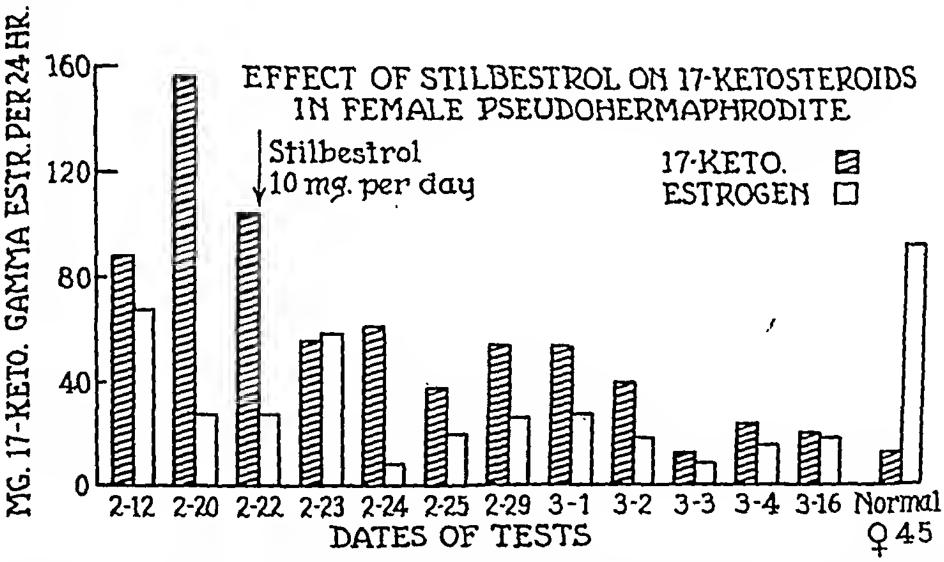


FIG. 5. Effect of stilbestrol medication upon urinary 17-ketosteroids and "estroids," as estimated microchemically, in a female patient aged 42, with adrenal virilism.

The female patient illustrated by the figure (age 42) suffered from generalized hirsutism and an enlarged clitoris, but no adrenal tumor. The internal genitalia were those of the female but the ovaries contained only sparse follicles. As shown in Figure 5, the continuous administration of large doses of stilbestrol caused a definite lowering of the 17-ketosteroid excretion, and thus an increase in the antithetical ratio. The urinary "estroids," however, failed to change significantly. One is reminded of the fact that the administration of methyltestosterone does not increase urinary 17-ketosteroids. Indeed, parenthetically, a slight fall (33) has been reported.

The effect of medication with testosterone, however, is readily detected in a rise of 17-ketosteroid excretion in appropriate cases. For example, a 21-year-old eunuchoid man showed a 17-ketosteroid excretion of only 7.5 mg. per 24 hours. With 150 mg. of testosterone propionate weekly injected intramuscularly, his excretion rose to 17 mg. daily. On the other hand, a 40-year-old woman, two years after oöphorectomy for mammary cancer with metastases, excreted only 4 mg. of 17-ketosteroids daily; and after

TABLE 2. RELATIONSHIP BETWEEN MICROCHEMICAL DETERMINATION OF "ESTROIDS" AND BIOLOGICAL STATUS

Patient	Age Yrs.	"Estroids"(E) micrograms /24 hrs.	17-Keto-steroids (A) mg./24 hrs.	Ratio E÷A
Pseudohermaphrodite				
B	5	129	21	6.1
Simple familial hirsutism				
C.M.	27	30	8.5	3.5
A.C.	28	18	8.3	2.2
H.J.	43	94	7.4	13.0
Adrenal hyperplasia				
M.F.	17	29	63	0.5
S.A.B.	25	39	48	0.8
E.B.	41	29	99	0.3

300 mg. of testosterone propionate weekly, her urinary level rose only to 6 mg. What did the cancerous patient do to the large amount of testosterone?

III. **Adrenal contribution.** That the adrenal cortex contributes to urinary 17-ketosteroid excretion is well recognized (34). Although estrogens have been isolated from adrenocortical tissue (8, 9), it is less well known that such estrogen-like substances may contribute to the urinary "estroids." As indicated in Table 2, however, in a case of true pseudohermaphroditism (kindly referred by Dr. Lawson Wilkins) a remarkably high microchemical value for "estroids" was observed. This precocious child of five years exhibited a urogenital sinus, but no tumor. The abnormal urinary chromogen indicated the presence of a substance with the chemical solubilities of estriol by the procedure of Bachman and Pettit (10) and a low biological estrogenic activity. In the table, the data for this youngster are contrasted with values for older females of two clinical types, i.e., simple hirsutism and adrenal hyperplasia (or metaplasia). The data suggest that

certain clinical syndromes involving hirsutism manifest characteristic antithetical E/A ratios.

Nevertheless, in certain syndromes involving the adrenal cortex both the "estroids" and the 17-ketosteroids may be elevated. If both are elevated to the same degree, the antithetical ratio may be normal.

IV. **Metabolic degradation products.** Among many others, Moore (27) pointed out that the discrepancy between bioassay for urinary androgens and the microchemical value for 17-ketosteroids was due to the low androgenic activity of the several end-products of testosterone metabolism, including their congeners of adrenal origin. According to Gallagher's scheme (6), testosterone is degraded to androsterone, which exhibits only one-third to one-tenth of the biological potency of the precursor. Testosterone also yields etiocholanolone (trans, alpha) and isoandrosterone, the biological potencies of which lie between 1 per cent and 5 per cent of the original testosterone. Equally impotent is dehydroisoandrosterone, presumably of adrenocortical origin. When a mixture of these substances (as extracted from urine) is assayed biologically, the androsterone molecules are weighted more heavily than the etiocholanolone, because the latter has lost most of its potency. Nevertheless, it might well be argued that the inactive molecules are more important because they represent androgenic material which has played its part in bodily metabolism. Indeed a long controversy has been waged over the question whether it is more significant to count "live bullets" or "discharged cartridge shells," to quote a widely used analogy. At least the chemical determination of 17-ketosteroids (4) weights all fractions equally, molecule for molecule, without regard to their final loss of potency.

Much the same problem presents itself for the urinary "estroids" because, as in the case of the androgens, the urinary extracts yield a mixture of partially or completely inactivated hormones. As in the case of the urinary "androgens" also, these metabolic products exhibit a wide range of potencies. In fact, the relative activities of estradiol, estrone and estriol may be regarded as approximately 10:3:1. These values vary widely, however, according to the biological procedures employed. Moreover, the biological behavior of known mixtures cannot always be predicted from the established activities of the individual constituents of such mixtures. A further complication is presented by the potential estrogen described tentatively by Smith and Smith (7) as a lactone. This substance is activated biologically by treatment with zinc and hydrochloric acid. It is interesting that the same treatment increases the microchemical values for urinary "estroids" from twofold to fourfold!

The use of the zinc reduction has two advantages: a) it increases the

total chromogen, thus making for more accurate readings; and b) it salvages certain urines which would be hopelessly contaminated with melanin-like pigment. For example, from 6-hour aliquots of a certain urine the following approximate *relative* amounts of interfering pigment (expressed in color-units) were derived: after simple hydrolysis and extraction, 2000+; after zinc reduction, 1054; after zinc reduction plus clean-up, 268. Inasmuch as the workable range of background is approximately 100 to 400, it is clear that by the use of zinc a result was obtained which otherwise would have been lost.

The advantage of Kober's reagent is that it registers the three urinary estrogens, molecule for molecule, without reflecting any loss of biological

TABLE 3. COLOR DEVELOPED BY PURE ESTROGENS* (at 130°C.)

Time in Minutes	Estrone	Estradiol	Estriol
6	19	23	0, 0, 3
6.5	21, 5, 23	22, 22.5	
7		23	
9	29.5, 31.5	25	7
9.5	33	26	
10			14, 14
10.5	31.5, 32	25, 27	12.5
11	32	27	18, 18
13	33, 33	25, 25	21
15	34	26	26. 26
17	34, 33	23, 26	25
19			37
20	30.5		35, 42, 42
25			40
30			44

* Color units developed by 25 micrograms of estrogen reacting in 4 ml. of Kober's reagent mixture.

potency. This fact was pointed out by Venning (35). In the present investigation, however, it was learned that the time of heating must be regulated carefully lest the estriol fraction develop a disproportionate degree of color. This fact is illustrated in Table 3. It will be observed that in about 20 minutes at 130°C. estradiol and estrone reach maximum color, which is slightly less for estradiol. The color for estriol, however, continues to deepen with time. In the procedure used in this study as little as 2 micrograms of estrone can be detected by the naked eye if interfering chromogens are eliminated.

An example may be cited. In pooled urines from U. S. Army personnel,

TABLE 4. OVER-ALL RECOVERY OF CRYSTALLINE ESTRONE ADDED TO URINE (6-HOUR ALIQUOTS) AND HYDROLYZED AT 100°

Estrone Added micrograms	Estrone Recovered micrograms	Per Cent Recovery
5	5	90
5	4	80
5	3	60
5	2	40
5	2	40
5	2	40
		Av. 58
10	8	80
10	8	75
10	8	75
10	8	75
10	6	60
10	6	60
10	6	60
10	4	40
10	4	40
10	3	30
10	2	20
10	2	20
10	0	0
		Av. 49
20	17	85
20	12	57
20	10	47
20	9	45
20	9	45
20	9	45
20	9	42
20	8	40
20	5	25
20	5	22
20	5	25
		Av. 44
30	15	50
50	21	42
		Av. 46
Grand Average		49%

the authors found a daily excretion of "estroids" corresponding chemically to approximately 11 micrograms of estradiol. By a modified Thayer-Doisey (36) bioassay procedure, however, the result was only 3 micrograms. Moreover, in gynecomastia based on malnutrition (26) the authors found even lower values by both methods; but the antithetical E/A ratio was normal. In gynecomastia, it seemed important to exclude the possibility that large amounts of highly *inactivated* estrogen were present in the urine. Therefore the microchemical procedure seemed particularly important in this latter instance, because it revealed a low "estroid" excretion in the presence of abnormal mammary hyperplasia.

V. Technical problems in methodology.

17-KETOSTEROIDS: In the microchemical determination of the urinary 17-ketosteroids, two major technical difficulties plague the investigator. These are:

a. the quantitative recovery of the 17-ketosteroids in a partially purified state; and

b. the elimination of extraneous pigments or interfering "background" during the final colorimetric procedure.

In the case of the 17-ketosteroids one widely used chemical method (20) is based on an optical correction for "background," whereas another method (4) involves the removal by extraction of the extraneous pigments. Both

TABLE 5. RECOVERY OF CRYSTALLINE ESTRONE FROM CLEAN-UP PROCEDURE

Estrone Added micrograms	Estrone Recovered micrograms	Per Cent Recovery
10	12	120
10	10	100
10	8	80
10	7.5	75
10	3.5	35
15	5.5	33
20	24	120
20	13	65
20	8	40
30	38	126
30	30	100
Grand Average		81%

of these obstacles are present in the microchemical determination of urinary "estroids," and both devices have been used to reduce the interfering pigments.

"ESTROIDS": *a. Quantitative extraction.* With respect to the recovery of urinary "estroids," the problem is essentially the same as in bioassay. In both procedures it is necessary to hydrolyze the conjugated estrogens and to separate them from a large mass of undesirable material. Under routine laboratory circumstances the recovery of added estrogen (i.e., a female complement introduced into male urine) may approximate 50 per cent. In Table 4 are given a series of recoveries from male urine "spiked" with

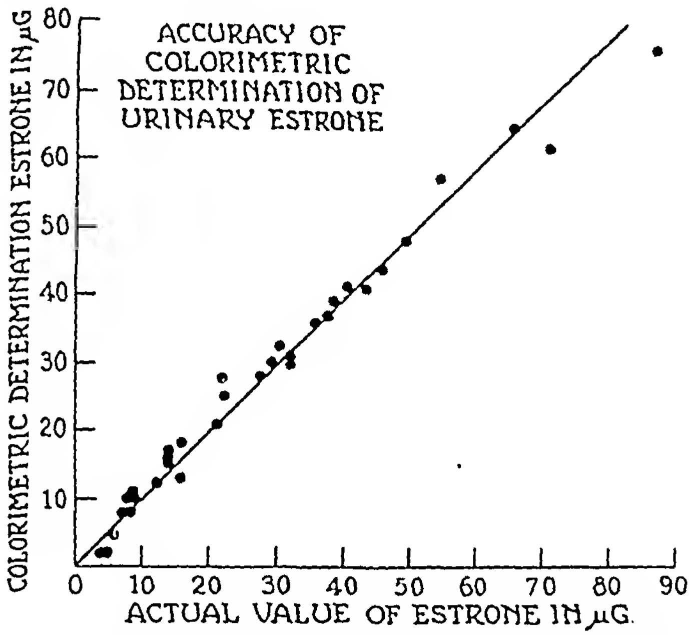


FIG. 6. Comparison of observed and theoretical values obtained by means of the nomogram shown in Figure 1.

known amounts of estrone. It should be noted that the over-all recovery of approximately 50 per cent can be broken down according to the individual stages of the procedure as follows:

1). Initial hydrolysis and extraction, separation and concentration of "estroids," 39 per cent loss.
2). Clean-up procedure to remove interfering brown pigment, 11 per cent loss. This loss is shown in Table 5.
3). Final colorimetry, 0 ± 3 per cent loss.

To those scientists who refuse to think in terms of a sliding scale when dealing with microanalysis this poor recovery will seem discouraging. It is clear at present that only relative values can be determined for urinary "estroids," whether bioassay or a microchemical procedure is used. Nevertheless, as a practical aid in improving extraction procedures, the colori-

metric estimation has distinct advantages over bioassay, in both accuracy and the amount of labor involved. The accuracy of the final colorimetric procedure is illustrated in Figure 6.

b. Elimination of interfering pigments. The amount of interfering background pigment varies widely from day to day in the same human subject. Indeed, an occasional urine specimen is encountered which is hopelessly "contaminated" with undesirable background pigment, and must therefore be discarded. Possibly a rigid control of diet and medication might reduce this unwanted chromogen to low values. The extraction procedure based on differential polarity removes much black tar and often over 90

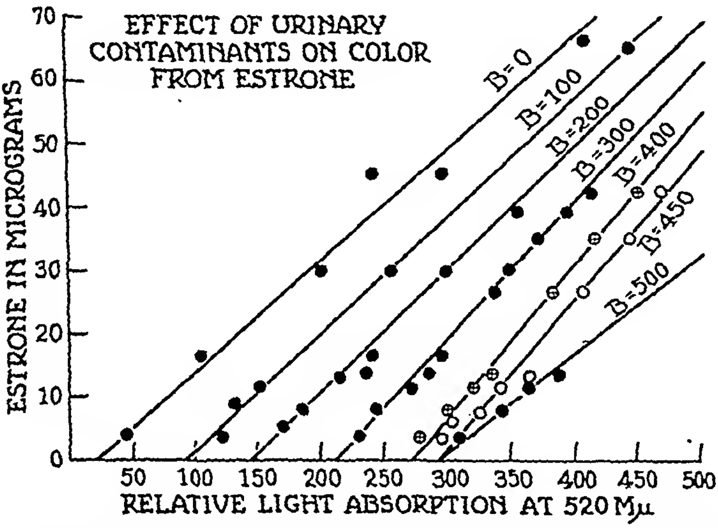


FIG. 7. The relation of extraneous "background" pigment to the photoelectric estimation of estrone. Each line represents a certain concentration of "background" as measured through a blue filter (No. 420).

per cent of the dark interfering pigment. Even under the most favorable circumstances, however, it has not been possible to extract completely the brownish color without a serious loss of the true vermilion of the estradiol-like chromogens.

Therefore, after the bulk of this material is gone, an optical correction is requisite, as shown in Figure 7. This correction may be applied algebraically at considerable cost of time and labor. It is more conveniently incorporated in a nomogram, such as that shown in Figure 1. This chart is based upon the tacit assumption that the composition of the undesirable pigment is invariable. As in the case of 17-ketosteroids, this assumption is not strictly true. Indeed, the method of Cahen and Salter (4) for 17-ketosteroids was developed in order to side-step this difficulty. In the case of the

"estroids," however, the assumption must be faced at present. Although doubtless introduces some variability into the data, there is no eviden from recovery experiments that average figures are altered appreciably l the optical correction. In short, until the recoveries of estroids from cru urine are much better than at present, the present optical technique adequate (although unsatisfactory).

Of course if a sensitive spectrophotometer is available, more accura results can be obtained and the nomogram can be dispensed with. The al sorption curve of the "background pigment" will vary somewhat with tl extraction and clean-up procedures used. A suitable study of its optic behavior, however, will reveal a wave band which will accentuate tl difference between the brown and the vermilion colors (e.g., at approx mately 520 mμ). As suggested by Stevenson and Marrian (37), tl vermilion pigment can be destroyed by heating and the residual backgroun read afterwards. Alternatively, readings may be made at two wave lengtl (e.g., 430 and 520 mμ, after *precise* determination under the individu circumstances), and the concentration of vermilion pigment may then l estimated by algebraic calculation or with the aid of a nomogram.

SUMMARY

The microchemical determination of urinary "estroids" bears man analogies to the determination of 17-ketosteroids. Each procedure raise similar questions, both physiological and technical. A tentative micr chemical procedure for urinary "estroids" has been mentioned and it application in selected clinical instances has been described. It is empha sized that at the present time only relative significance can be expected c the values so obtained. Many obstacles militate against absolute accurac for "estroid" excretion, but similar obstacles exist for the estimation o 17-ketosteroids.

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ALKALINE PHOSPHATASE AND GLYCOGEN IN HUMAN ENDOMETRIUM

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BECAUSE of the morphogenetic properties of the sex hormones, the histologic study of accessible sex organs has been demonstrated to be very useful in the evaluation of genital functions. However, we believe that the histochemical study of such organs and tissues promises a better understanding of the many problems involved.

So far as we know, glycogen and lipids are the only substances which have been systematically investigated by means of histochemical reactions during the human endometrial cycle. Except for the excellent work by Wislocki and Dempsey (1) on the mucosa of the pregnant uterus, phosphatases have not been studied in the human endometrium. It can be stated that, to date, only observations on the *location* of phosphatase have appeared in the literature, such as the report of Kabat and Furth (2), who demonstrated the absence of phosphatase in the myometrium and its presence in small amounts in the epithelia. The relation between this enzyme and estrogen action in mouse endometrium has been shown by Atkinson and Elftman (3) and mentioned by Gomori (4, 5).

The purpose of this paper is to report our findings on this subject and to indicate some problems that arise from the possible relationship between alkaline phosphatase and glycogen. It is well known that a mechanism of phosphorylation catalyzed by phosphatases exists in the metabolism of glucides and lipids (Dempsey and Wislocki (6)), which in all probability plays an important role in the nidation of the ovum and the endocrine functions related to the endometrium.

METHODS

The endometrial specimens obtained by biopsy were immediately fixed in 96 per cent alcohol and often refixed in alcohol-formol-picric mixtures (unpublished data). For the study of glycogen, this method of fixation has proved to be very helpful when carmine dyes are used.

Carmine, the leuco-fuchsin reaction for aldehydes, and the silver methods devised by Mitchell and Wislocki (7), Gomori (8), and one of us (9), have been used for the demonstration of glycogen. Digestion in saliva and adequate pretreatments were employed in order to rule out other substances. Because of the advantages of the silver procedures, these have been favored in the present investigation.

For the demonstration of alkaline phosphatase, Gomori's technic (4)

as modified by Wachstein (10) has been followed. Some slight variants however have been introduced in certain cases; as for example, shortening of the incubation period under higher temperatures. Some functional differences could be shown by this means, such as the frequent presence of the enzyme in the stroma, near the glands and capillaries, and its presence in extensive cytoplasmic zones that otherwise would have been ignored. Hematoxylin-eosin was used for the "control" slides. Counterstaining of glycogen and phosphatase reactions was generally omitted. Most of the time, both glycogen and phosphatase reactions were done on each specimen.

RESULTS

Four categories (two of them subdivided in 3 groups each), as judged by the morphological patterns and the clinical data available, have been composed from 73 specimens studied. The following key was used for the interpretation of the reactions obtained.

Alkaline Phosphatase

0	Negative reaction
+	Positive reaction in capillaries only
++	Positive reaction in nucleoli also
+++	Positive reaction in nuclei
++++	Positive reaction in cytoplasm

Glycogen

0	Negative reaction
+	Very small granules
++	Coarse granules
+++	Small masses
++++	Large amounts

Although arbitrary and relative, the above scale made possible a quantitative estimation of the histochemical reactions obtained. This is shown in Table 1 for 68 specimens. Three specimens of decidua and two showing endometrial tuberculosis were also studied.

Since most of the samples came from gynecological patients, abnormalities prevailed in the whole group. Moreover, a certain selection was made in order to secure several morphological types. A brief description of our findings follows:

A. *The distribution of alkaline phosphatase*

During the estrogenic phase epithelia and capillaries are rich in phosphatases and this seems to increase in proportion to the estrogen stimulus (see Table 1). Gland cells and epithelial surface cells show the enzyme in all their structure, both nuclei and cytoplasm (Fig. 1). As the cycle advances, particularly from the midcycle on, the cytoplasm and the nuclei show a less intense reaction. Thus during the transitional phase, although the

enzyme is still abundant, especially in the epithelial surface, subnuclear vacuoles appear. The nuclei give a positive reaction mainly in the nucleoli and very often just the apical portion of the cytoplasm shows an intense reaction. This is also shown by secretion in the gland lumen. The epithelial surface remains unchanged for a longer period of time. During the progestational phase only secretions, capillaries and sometimes nucleoli produce a positive reaction (Fig. 2); still later, near menstruation, only the capillaries. Practically no reaction is present during menstruation although some variations exist in different specimens. The reaction in the stroma is almost always negative and only during the progestational phase have we seen positive reactions in the nucleoli and certain decidua-like periglandular and perivascular cells.

TABLE 1. RESULTS OF REACTIONS FOR ALKALINE PHOSPHATASE AND GLYCOGEN IN HUMAN ENDOMETRIUM*

Endometrial Type	Alkaline Phosphatase			Glycogen		
	No. of cases	Average†		Average†		No. of cases
Estrogenic						
Hypoestrogenic	9	0/+	(0.44)	+/++	(1.7)	9
Normal	4	+++/++++	(3.5)	+/++	(1.5)	4
Hyperestrogenic	5	+++/++++	(3.6)	+/++	(1.8)	6
Transitional	6	++/+++	(2.4)	++/+++	(2.2)	5
Progestational						
Hypoprogestational	20	+/++	(1.6)	++	(2.1)	19
Normal	10	0/+	(0.66)	+++/++++	(3.2)	9
Menstrual	8	0/+	(0.62)	+/++	(1.6)	8
In Thyroid Disease	6	++++	(4)	+/++	(1.6)	5
Total	68					65

* For key, see section on "Results."

† The figures in parentheses are an attempt to express quantitatively the averages of the reactions obtained for each group.

The main characteristics in the definitely abnormal cases are irregularities of distribution and increase, decrease, or even absence of phosphatase paralleling the degree of endometrial development. An important exception was found in the somewhat paradoxical picture of the endometrium in hypothyroidism (11, 12), which we hope to discuss in a separate paper.

Up to the present time only three deciduas have been studied. In two cases of spontaneously eliminated fragments we found negative reactions. This disagreement with the findings of Wislocki and Dempsey (1) can be explained, we believe, by the endocrine abnormalities of the patients, who showed a tendency toward abortion and bleeding.

The third specimen unexpectedly obtained by biopsy was very rich in phosphatase. The almost absolute absence of glycogen in all three specimens may support this hypothesis.

In two cases of endometrial tuberculosis we did not find any enzymatic activity in the giant cells.

B. The distribution of glycogen

The amount of glycogen present in the endometrium during the estrogenic phase varies from traces to coarse granules situated basally or surrounding the nuclei of the

FIG. 1. Alkaline phosphatase during the estrogenic phase (×350).

FIG. 2. Capillaries and secretion are positive for alkaline phosphatase during the progestational phase (×250).

glandular and epithelial cells (Fig. 3). It is even possible to see such granules in the process of being secreted. During the transitional phase, morphologically considered as the ovulatory phase (although we believe it is not necessarily so), the subnuclear and often all the perinuclear zones have been found loaded with glycogen. Later on, as the progestational phase advances, the epithelial cells, especially the gland cells, discharge into the apical portion of the cytoplasm or into the gland lumen. The stroma shows more and more granules and cytoplasmic masses of glycogen. During menstruation the polysaccharide seems to be washed out and the amount diminished. In some instances a considerable quantity was found in the vascular walls.

Specimens poorly transformed or irregularly matured by progesterone showed scanty and unevenly distributed glycogen tending to be confined inside the gland cells as described by Hughes (13). On the other hand, in one case (not tabulated) from a luteal ovarian cyst there was a large amount of glycogen in the numerous glands present. A fairly large quantity was contained in hyperplastic endometrium (Fig. 4) without morphological signs of progestational response. In Figure 5 is seen a curve which schematically

expresses different levels of both substances during a more or less theoretical cycle[1] and during some of its hormonal abnormalities.

DISCUSSION

We believe that the results obtained are fairly consistent. With respect to our findings in the investigation of human endometrial glycogen they coincide in general with the data reported by others (13, 16, 17, 18, 19, 20).

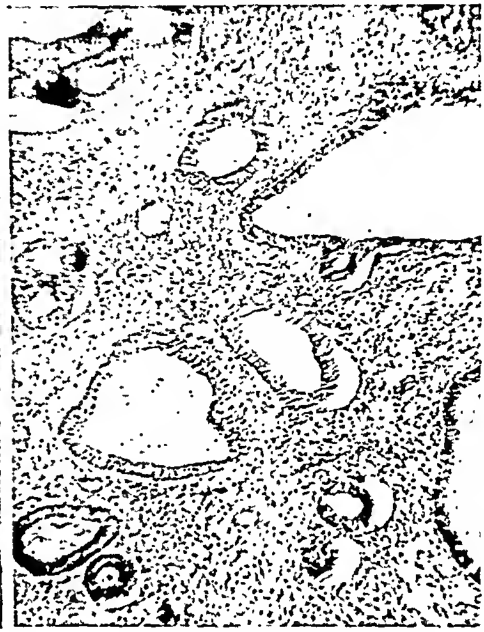

FIG. 3. Glycogen in a morphologically estrogenic specimen (Arzac's silver method) (×720).

FIG. 4. Cystic hyperplastic endometrium showing glycogen granules and small masses basally and around the nuclei (Arzac's silver method) (×100).

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[1] We have said a "more or less theoretical cycle" because it is well known from biologic determinations that the hormonal levels of estrogen and progesterone during the *real* cycle oscillate in a way essentially similar to the one expressed in the abscissa of our graph (excepting the HE point). The estrogen curves of D'Amour (14) and of Markee and Berg (15) for example show a relative hypoestrogenic status at the beginning compared to more advanced epochs of the cycle, near ovulation time. Furthermore the points hE and hL of our graph correspond to reactions observed in specimens taken in the early proliferative and early secretory phases. In these, the histological aspects correspond to about the seventh to the ninth days and the twentieth to the twenty-second days of the normal cycle, respectively. On the other hand, the numbers in the ordinate are not strictly subjective values but an attempt to express quantitative averages (see Table 1).

The premenstrual peak is particularly pronounced, whereas other peaks are not quite so definite. However, some facts often forgotten or debated (Moricard (21), Beclere (22)) deserve to be emphasized, namely, the existence of rather large amounts of glycogen in purely estrogenic endometria (Fig. 3), showing either morphologic hypoplasia or glandular cystic hyperplasia (Fig. 4). Of course it is not unusual to obtain positive reactions

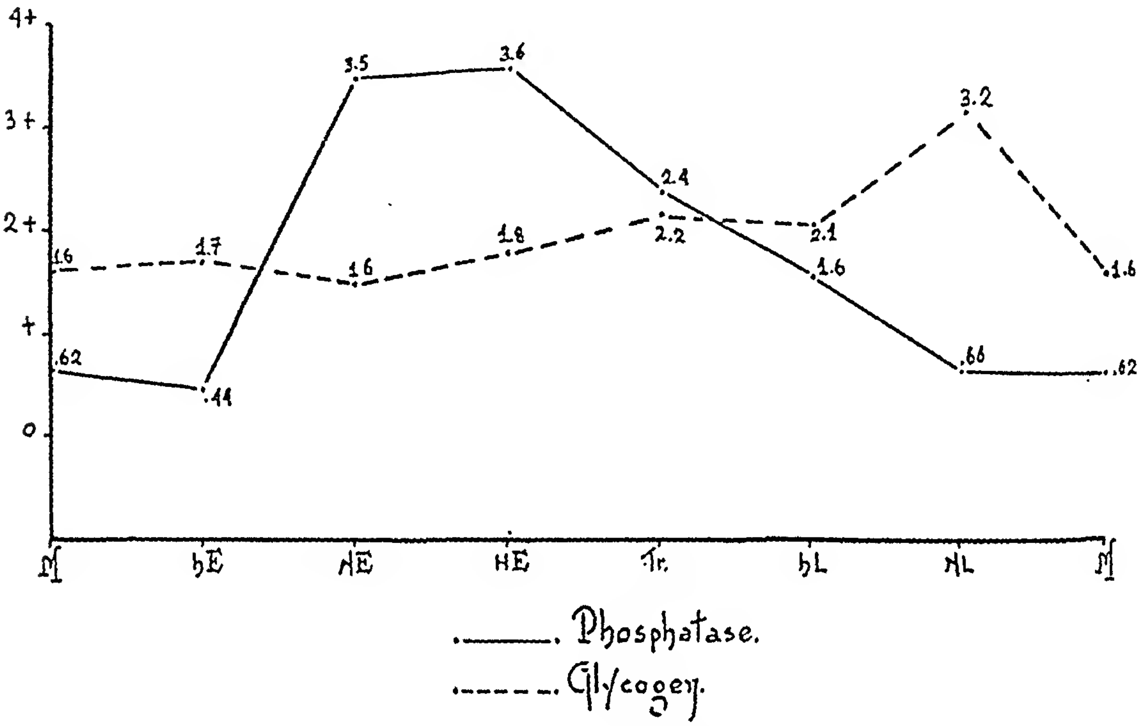

FIG. 5. Graph composed of the averages of the estimated values for alkaline phosphatase and glycogen obtained from 68 specimens (see Table 1). M (menstruation); hE (hypoestrogenic); NE (normal estrogenic); HE (hyperestrogenic); Tr (transitional); hL (hypoluteinic); NL (normal luteinic).

in "mixed" specimens with or without hyperplasia. It seems important, however, to stress the fact that in some cases (such as those which show a hypoestrogenic condition morphologically) estrogen alone seems sufficient to stimulate the appearance in and even a slight discharge of glycogen from the glands of the human uterine mucosa. In this respect the situation seems to be identical with the one in macacus rhesus (Hisaw (16); Overholser and Nelson (23)) and in the rat (Boettiger (24)). This has escaped the attention of many gynecologists because of a lack in the past of histochemical procedures as efficient as the silver techniques previously mentioned. In fact Zondek and Stein (20), and Spyker and Fidler (19) were able to demonstrate glycogen during the estrogenic phase by chemical analysis but not histochemically.

From another point of view our results are in disagreement with the

findings of others. Thus Spyker and Fidler (19) did not find glycogen in the stroma whereas in our specimens we were able to demonstrate considerable amounts of it. Perhaps the explanation advanced for similar findings by Hughes (13) would apply in this instance, as many of our specimens came from cases of sterility. Nevertheless, it must be pointed out that most of our morphologically normal progestational specimens revealed glycogen in the stroma. It is interesting also that morphologically pure estrogenic specimens, even those premenstrual biopsy specimens showing hypoplasia, had somewhat larger amounts of glycogen than those from the proliferative phase of normal cycles. A lack of parallelism between morphology and chemical status in such instances may perhaps be more frequent than is thought.

Concerning phosphatase it seems quite clear that progesterone has an effect upon this endometrial enzyme not established, so far as we know, by previous investigators. Thus the only mention that we have found in current literature is the one made by Atkinson and Elftman (25) who state that the circular muscle layer of mouse uterus shows a disappearance of the reaction when the animal is treated with progesterone and that the results elicited by estrogen and progesterone are the same as after estrogen alone. Our data show on the contrary that, in human beings at least, the responses are quite different from each other and that the more advanced the progestational phase, the smaller the amount of phosphatase present in the endometrium; whereas the opposite happens during the estrogenic phase.[2] This cannot be attributed to a lack of estrogenic action during the luteal phase since it is well known that the quantity of this hormone increases during the second half of the cycle.

The relationship between alkaline phosphatase and glycogen is very interesting. It seems quite clear that the amount of each, particularly during the progestational response, shows an inverse ratio. This suggests that glycogen is formed at the expense of phosphatase, which is in agreement with the phosphorylation theory of glycogenesis. In fact Wislocki and Dempsey (1) have reported that whenever a process of glycogen deposition is found, a barrier or a bed of phosphatase is always present or interposed between circulating blood and the place in the tissue where glycogen is deposited. Since only the endometrium of pregnant women was studied by them, they reported the presence of phosphatase only in the capillaries (at the beginning of pregnancy) and they supposed that this barrier was sufficient explanation for the appearance of the polysaccharide

[2] After this paper was written our attention was called to a very recent publication by Atkinson and Engle (26) who, in a smaller number of cases, found essentially the same results as ours in monkey and human endometrium.

in different places in the mucosa. Our results show however that in human endometrium a bed, in addition to a barrier, of phosphatase is found sometime during the cycle, preceding the deposition of glycogen in the particular cells involved.

If we consider now what happens under the influence of estrogenic action it is evident that glycogen, although in small amounts, is synthesized in the presence of large amounts of the enzyme; and that in certain cases (during the late estrogenic and the transitional phase) both are present in rather large quantities. This suggests that on the one hand the presence of sugar and phosphatase is not sufficient for the establishment of a full catalytic process of polymerization; and on the other hand, that the whole process is gradually and not suddenly established.[3]

It appears safe to assume that progesterone is the factor involved in the activation of the catalytic reaction, evident during the progestational phase. Since, however, this reaction, although less intense, can be evoked under the influence of estrogen alone, several hypotheses can be advanced tentatively: (a) estrogen and progesterone have, from this particular point of view, only a quantitative and not a qualitative difference; (b) progesterone is secreted during other than the second half of the cycle; (c) metabolites, progressively produced under estrogenic action, progressively activate the catalytic reaction; (d) several enzymes act at different epochs of the cycle. For the time being we have no conclusive evidence to support any one of the mentioned hypotheses to exclusion of the others, so that it is more than likely that several factors are involved in the production of the phenomena described. Of course the role of phosphatase in the process of growth cannot be excluded, since it seems to be involved in the synthesis of nucleoproteins. In fact, we found a very intense reaction, together with the presence of vitamin C granules, in such an actively growing tissue as a highly malignant endometrial adenocarcinoma (not tabulated). On the other hand, the part phosphatase can play as a respiratory enzyme is another possibility. In this respect the observation that a maximum response is found in the endometrium of those patients in whom thyroid function is subnormal, deserves careful consideration.

It is obvious that routine studies of the two histochemical reactions here mentioned are highly desirable since they can throw a great deal of light on the physiology of the uterine mucosa. This may be particularly true in cases of sterility which show a definite tendency toward phosphatase-glycogen imbalances, either because the enzyme appears increased due to poor utilization for glycogen synthesis, or because both are dimin-

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[3] The facts just reported by Zondek and Hestrin (27) give support to this point of view.

ished when glycogen synthesis is decreased due to scarcity of the enzyme. Perhaps anovulatory menstruation and other "non-menstrual" bleedings will be differentiated more clearly by such studies.

SUMMARY

1. Alkaline phosphatase and glycogen have been studied histochemically in seventy-three biopsies of human endometrium.

2. It was found that the amount of alkaline phosphatase demonstrable in the endometrium increases during the proliferative estrogen phase of the menstrual cycle, whereas it decreases during the progestational phase and disappears during menstruation. It is abundant in the endometria of patients with gynecological disorders associated with hypothyroidism.

3. With the silver techniques used, glycogen could be histochemically demonstrated not only during the progestational phase but also during the proliferative phase; and in hypoplastic as well as in hyperplastic estrogenic specimens. The results with these procedures agree in a general way with the quantitative biochemical data so far reported in the literature available to us.

4. The results obtained are discussed and the suggestion made that the histochemical study of the endometrium is highly desirable, particularly in cases of sterility.

ACKNOWLEDGMENT

We are very indebted to Dr. Alfonso Alvarez Bravo, Dr. Carlos D. Guerrero, Dr. Alejandro Otero and Mr. Sylvester Frenk for the specimens submitted.

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USE OF GLOBIN INSULIN IN ADDISON'S DISEASE ASSOCIATED WITH INSULIN-SENSITIVE DIABETES

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IN 16 cases of coexisting diabetes mellitus and Addison's disease so far reported in the literature, insulin sensitivity has been a frequent occurrence and has presented a difficult therapeutic problem. In three of the cases reported, diabetes developed after the onset of Addison's disease (1, 2, 3).

Simpson's (1) patient had been treated elsewhere with epinephrin injections. Following treatment with adrenal cortical extract, hyperglycemia and glycosuria developed within one month. Insulin was administered. This resulted in severe hypoglycemic reactions from even small doses. The patient eventually came to autopsy and the diagnosis of Addison's disease was substantiated.

The patient described by Rhind and Wilson (3) was treated with desoxycorticosterone for one year and then developed diabetes. This individual was the only one of fifteen who was apparently not extremely sensitive to insulin. The patient was taking 60 units of insulin per day and eventually expired in a hypoglycemic convulsion.

Thorn and Clinton (2) reported a case of Addison's disease treated with desoxycorticosterone for three years prior to the onset of diabetes. Small doses of regular insulin were given. By this method, severe reactions, such as might be induced by protamine zinc insulin, were avoided.

In the remaining thirteen cases which were reported, the Addison's disease developed either concurrently with diabetes (4) or after the diabetes (4–13).

Rogoff (14) reported a patient, who, before coming under his care, had had the adrenals denervated in order to alleviate diabetes. The diabetes, prior to operation, had been controlled with diet and insulin. The patient developed Addison's disease. His insulin requirement decreased and he became sensitive to insulin. The procedure was condemned by the author.

Armstrong (15) reported a patient who had diabetes associated with Addison's disease and whose insulin requirement prior to the development of Addison's disease was 56 units. After development of Addison's disease his requirement dropped to 8 units daily. The patient was sensitive to

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insulin and developed hypoglycemic reactions. In this case adrenal cortex extract (Eschatin) produced glycosuria and hyperglycemia.

CASE HISTORY

In our patient, a colored female, aged 30, Addison's disease was diagnosed on her first admission, December 7, 1944. Complaints: loss of energy and weight, anorexia, nausea and diarrhea. She also stated that her skin had become darker and that pigmented areas had appeared on her gums. She was extremely emaciated and asthenic. Her blood pressure was 70/40. Blood chemistry showed serum sodium, 126.9 mEq./L, and potassium, 4.75 mEq./L. Two days later the potassium was 5.1 mEq./L. Glucose was 66 mg. per 100 cc. and CO_2 combining power, 20.4 mEq./L. Hemoglobin was 14.6 Gm., red blood cells 4.46 million and white blood cells 7,200. The differential count was normal. The water excretion test (16) showed the following: part I, positive; part II, 4.9 (normal 25+).

The Addison's disease was brought under control by glucose, sodium chloride, adrenal cortical hormone and desoxycorticosterone. The natural hormone was continued for seventeen days and a total of 170 cc. (425 rat units) was given. The symptoms were relieved. The patient gained strength and her weight varied from 95 pounds to 103 pounds.

At first, 15 mg. of desoxycorticosterone acetate were required daily but at the end of nine months she was able to do light housework while taking 5 mg. every other day. At this time three 75 mg. pellets of desoxycorticosterone[1] were implanted beneath the skin. The patient's capacity for work remained good for a period of six months, after which she reentered the hospital complaining of weight loss (7 pounds), polyuria, excessive thirst, anorexia, and weakness.

Physical examination: Temperature 99°, pulse 74, respirations 20, blood pressure 70/50. Examination revealed an emaciated colored female with evidence of dehydration. Skin folds along the thighs and legs showed deeper areas of pigmentation. Pigmented areas were also present in the mucous membrane of the gums. Sibilant râles were heard in the upper lobe of the right lung, posteriorly. There were no significant findings in the heart or abdomen. There was no Babinski. Pelvic and rectal examinations were negative.

The first impression that the patient's condition was due entirely to undertreatment of the adrenal cortical insufficiency, was not substantiated by the laboratory findings.

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[1] Desoxycorticosterone pellets were supplied through the courtesy of Dr. Edward Henderson, Schering Corporation, Bloomfield, New Jersey.

Blood:

Serum sodium	305 mg. per 100 cc.; (132.6 mEq./L)
NPN	32 mg. per 100 cc.
Glucose (fasting)	346 mg. per 100 cc.
CO_2	47.5 per 100 cc. (21.3 mEq/L)
HB	12.4 Gm. per 100 cc.
RBC	3.98 million
WBC	7100

Cephalin-cholesterol liver function test—negative.
Thymol turbidity—1 unit.
Thymol flocculation—0.
Urinalysis—S.G. 1,020; albumin negative; glucose 4 plus; acetone positive; diacetic acid negative.
Skin test for hemochromatosis (17)—negative.
24-hour urine for hemosiderin—negative.

The patient's condition was not improved by supplementary desoxycorticosterone, 5 mg. daily, or by increased sodium chloride intake. Weight did increase after insulin was started. However, hypoglycemic reactions of unusual severity occurred without warning even from as small a dose as 5 units of regular insulin. The reactions were not always accompanied by perspiration. On several occasions the patient was found comatose in bed and unable to swallow. In each instance the onset of hypoglycemia was so rapid that disorientation or loss of consciousness developed before the patient could summon help although she was in a three-bed ward. In spite of the reactions, her general condition improved. She gained weight and strength. On March 13, 1947, her temperature rose to 102° and there was evidence of bronchopneumonia. Penicillin, 25,000 units every three hours, was given and the temperature gradually fell to normal within four days (Fig. 1).

On March 31, 1947, two 75 mg. pellets of desoxycorticosterone were implanted and intramuscular injections were discontinued. In view of the severity of the insulin reactions, it was decided to attempt control of the diabetes without insulin. The patient was discharged on March 31, 1947, on a diet of protein 70 Gm.; fat 125 Gm.; and carbohydrate 200 Gm. She, however, did not regain her former strength and working capacity and by April 4, 1947, had lost weight from 89 pounds to 83 pounds. A 24-hour urine specimen on April 4, 1947 contained 70 Gm. of glucose. Readmission to the hospital was advised to institute insulin therapy.

On admission to the hospital May 13, 1947, the patient was continued on the diet of protein 70 Gm.; fat 125 Gm.; and carbohydrate 200 Gm.; and globin insulin, 4 units daily before breakfast, was started. This was gradually increased to 14 units daily. During the ten day hospital stay the

patient gained from 77 pounds to 89 pounds and no hypoglycemic reactions occurred. The 24-hour output of glucose in the urine fell to 20 Gm. The patient was discharged from the hospital on May 23, 1947 (Fig. 2).

In the past three months the patient has felt well, has done all her housework and gained weight to 96 pounds. The present insulin dosage is

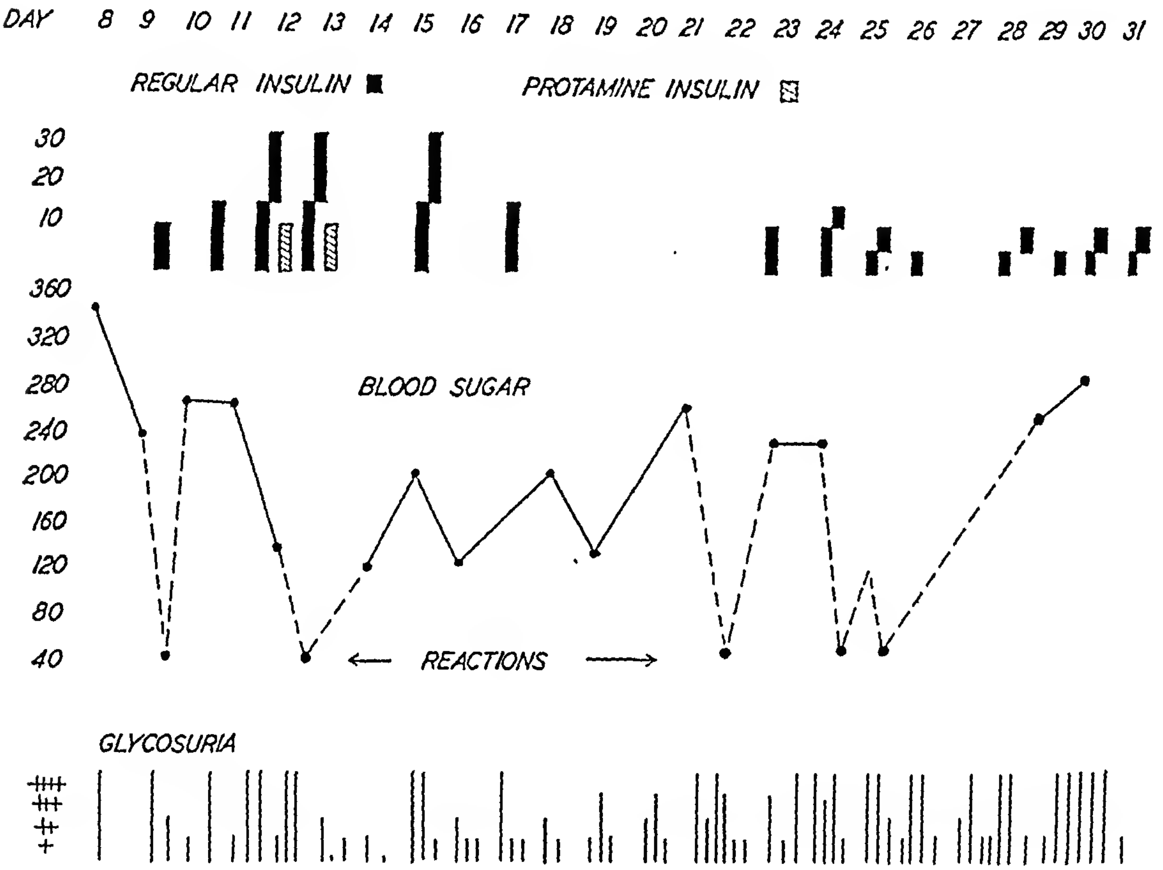

FIG. 1. Frequent severe hypoglycemic reactions during administration of regular insulin and of protamine zinc insulin in a patient with Addison's disease complicated by diabetes mellitus. (In this figure and in Figure 2, the ordinate numbers pertaining to insulin indicate the dose in units: those pertaining to blood sugar indicate mg. per 100 cc.)

12 units of globin insulin before breakfast. She has had one insulin reaction to this dosage. On July 12, 1947, the patient was brought to the hospital in hypoglycemic coma and was promptly revived by intravenous glucose. The preceding evening the patient had been moving furniture in preparation for redecorating her home.

On January 4, 1948 the urinary neutral 17-ketosteroids in twenty-four hours measured 0.5 mg. In the determination of the neutral ketosteroids the urine was hydrolyzed and fractionated according to Pincus (18); and

ketosteroids, separated by means of Girard's reagent (fraction 12), were determined by two-color spectrophotometry.[2]

COMMENT

In this patient, Addison's disease preceded the onset of diabetes mellitus by 2½ years. Three similar cases have been reported in the literature.

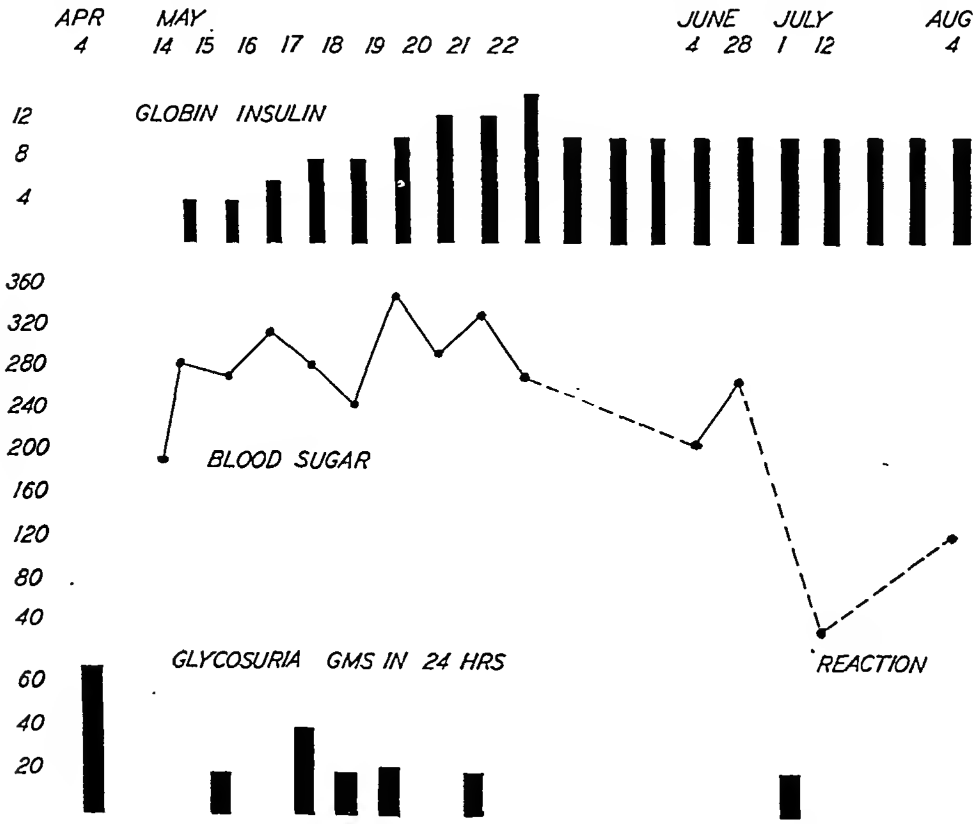

FIG. 2. Infrequent hypoglycemic reactions during administration of globin insulin in same patient shown in Figure 1.

Adrenal cortical hormone was administered only during the first seventeen days of therapy of the Addison's disease. Subsequently control was maintained with injections of desoxycorticosterone and later by pellets.

The onset of diabetes was abrupt and characterized by weakness, weight loss, polyuria and polyphagia. In spite of this and the high blood sugar and

[2] For this determination we are indebted to Mr. William T. Beher. Fellow in Biochemistry at the Research Institute of this hospital.

pronounced glycosuria, the patient did not have severe ketosis at any time. The blood CO_2 was only slightly decreased and diacetic acid was not found in the urine, although acetone was present.

The decision to use globin insulin resulted from the unsatisfactory and unpredictable results obtained from either unmodified or protamine zinc insulin. Protamine zinc insulin, with its maximum effect during the meal-free night hours, was obviously unsuitable and produced a severe hypoglycemic reaction during the night. Unmodified insulin also proved unsuitable, apparently because of its short and concentrated action. Globin insulin has a relatively slow action and the period of maximum activity coincides with the period during which the patient is taking food and is awake. For these reasons globin insulin seemed to offer the most promise for freedom from hypoglycemia. In this one case globin insulin apparently has been a more suitable therapeutic agent.

SUMMARY

In a Negress with Addison's disease followed by diabetes mellitus, globin insulin was the insulin of choice for treatment of the diabetes without resulting frequent severe hypoglycemic reactions.

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DIABETES IN A DWARF. A CASE REPORT

HERBERT SCHREIER, M.D. AND ISAAC LEWENTER, M.D.

From the Endocrine Clinic at Gouverneur Hospital, New York City

THE case presented is one of a juvenile diabetic who survived the preinsulin era. Her stunted growth gave rise to speculation on the "endocrine" stigmata of her dwarfism, whereas the starvation management of her diabetes in the preinsulin era was probably the main contributory factor. The course of her diabetes, complicated by hyperthyroidism, presents some points of interest.

CASE HISTORY

R.G., a 45-year-old white female, had no familial history of diabetes or dwarfism (Fig. 1). One older normally built sister suffered from rheumatoid arthritis; her rather

FIGURE 1

tall brother, from night blindness. The patient, the youngest of the three children, was born when her mother was 51 years old. She had an uneventful childhood, although

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she was sickly and could not follow a regular school curriculum. At the age of one year she had pneumonia. Menstruation started at eleven years, and was regular until November 1946, when the menopause ensued.

The diagnosis of diabetes was made at eleven years of age. Since this was in the pre-insulin era, the patient was subjected to prolonged hospital observation and starvation regimens at many hospitals. Insulin was administered to her for the first time in 1922, at the age of twenty, following diabetes of nine years duration.

The patient has been under observation in the diabetic clinic at Gouverneur Hospital since 1934, at which time the daily dose of insulin was 30 units.

In 1937, clinical evidence of hyperthyroidism developed, indicated by extreme nervousness, tachycardia, tremor of the hands and elevation of the basal metabolic rate to plus 32 per cent. The daily insulin dose had to be increased to 140 units. Thyroidectomy was recommended, and subtotal thyroidectomy was performed at Post-Graduate Hospital in January 1938. After an uneventful postoperative course, the patient was discharged from the hospital on a low caloric diet and on the same daily dose of insulin (140 units). On February 21, 1938, one month postoperatively, the B.M.R. was plus 15 per cent.

In April 1938 the patient started to gain weight, the hair on her head began to fall out, and she started complaining of paraesthesias. The B.M.R. fell, until on August 1, 1938 it was minus 1 per cent, and two weeks later minus 15 per cent. The insulin dose remained the same, 140 units daily.

Since 1938, the patient's attendance at the diabetic clinic at Gouverneur Hospital was more regular. The carbohydrate content of her diet through that period was 150 Gm. (Protein—60 Gm., Fats—60 Gm.). The insulin dosage administered up to date remains high: 70 units of protamine zinc insulin and 35 units of crystalline zinc insulin.

In January 1947 the patient started showing signs and symptoms of recurrent hyperthyroidism: loss of weight, exophthalmos, tremor, tachycardia (pulse rate 110/min.), a B.M.R. of plus 30 per cent, and two small nodules in the remaining thyroid. The RBC was 3,880,000; and the WBC, 8,505. Differential count: polymorphs—38 per cent, lymphocytes—49 per cent, monocytes—8 per cent, eosinophiles—4 per cent, basophiles—1 per cent. During treatment with propylthiouracil, pyridoxine, and feosol at the Post-Graduate Hospital Clinic, she responded very slowly; finally she showed a remission in June 1947. At present (Oct. 1947) she has a normal basal metabolic rate and is still on a maintenance dose of 25 mg. of propylthiouracil twice daily.

There has been no history of any insulin reaction or diabetic acidosis during the entire course of observation.

Physical Findings: Height—4 ft. 5 in.; weight—118½ lbs. Pronounced obesity with fat distributed predominantly in the breasts and trunk, and in the large abdominal apron. Hands and feet, particularly the fingers and toes, were short, small and thin. Hair distribution normal. Slight exophthalmos. Blood pressure, 120/80. Pulse rate, 80/min.

Essential Laboratory Findings: Blood sugar levels ranged between 200 and 300 mg. per 100 cc. The most recent report showed 200 mg. per 100 cc. The urine had never been found sugar free, although quantitative excretion of sugar in 24 hours had varied. The blood count was normal. Blood chemistry showed a non-protein nitrogen of 30 mg. per 100 cc.; free cholesterol, 159 mg. per 100 cc.; and cholesterol esters, 101 mg. per 100 cc.

X-ray study revealed a normal sella turcica with normal vascular markings. The suture lines appeared to have closed prematurely. Some prominence of convolutional markings was observed. The pineal body was not visualized. Long bones were normal.

DISCUSSION

Type of Dwarfism: In the differential diagnosis of the type of dwarfism in our case, we may, a priori, rule out some types of secondary dwarfism with characteristic features completely different from the signs shown by the patient. In this category belong osteopsathyrosis, achondroplastic dwarfism, and dwarfism due to renal rickets, congenital disorders or chronic infections.

The three types of dwarfism to be considered are: (a) primary, (b) pituitary and (c) diabetic dwarfism. Primary dwarfism involves a defect in the constitutional growth process on a hereditary basis (1, 2). This element cannot be found in our patient, who shows externally all the signs of a pituitary dwarf (3, 4), with one very important and decisive exception. She is sexually well developed; whereas the characteristic feature of pituitary dwarfism is infantilism of the primary sex organs and amenorrhea.

In physical appearance, diabetic dwarfs resemble pituitary dwarfs. The sella turcica, however, is normal, and sexual hypoplasia and amenorrhea are not present. Dwarfism in diabetics develops in childhood, not as a result of the diabetes as such, or because of other endocrine disturbances, but rather because of faulty control of the diabetes and malnutrition (5, 6, 7). Our patient developed diabetes long before insulin was discovered, and in the interim the disease was not well controlled. The presence of uncontrolled diabetes, accompanied by poor nutrition, probably caused retardation of growth. It would seem logical, therefore, to accept the diagnosis of diabetic dwarfism, due to uncontrolled diabetes in childhood (8).

Some Special Features of Diabetes in the Described Patient: Despite high doses of insulin there were no insulin reactions. Although she was a juvenile diabetic, the patient did not show signs of diabetic ketosis. The mildness of the diabetes in this young girl, if attributed to hypopituitarism, would imply Houssay's phenomenon in a human, but insulin resistance does not confirm this impression.

It is interesting that thyroidectomy changed neither the insulin requirement nor the clinical features of the diabetes.

SUMMARY

1. A 45-year-old diabetic dwarf, who had lived for nine years in the preinsulin era, has been presented. Hyperthyroidism developed as a complication in her thirty-fifth year.

2. In this type of dwarfism, endocrine factors other than the pancreatic diabetes were formerly over-stressed.

3. Response to insulin was unlike that of a juvenile diabetic in that there was no ketosis and no insulin reactions.

4. Despite the successful treatment of the hyperthyroidism, large doses of insulin were continuously required.

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PROGRAM OF THE THIRTIETH ANNUAL MEETING OF THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS

The Thirtieth Annual Meeting of the Association for the Study of Internal Secretions will be held in the Palmer House, Chicago, Illinois, June 18 and 19, 1948.

The scientific sessions will be held in the Red Lacquer Room and registration will be on the fourth floor just outside the Red Lacquer Room. The Annual Dinner will be held in the same room on Friday, June 18th at 7 P. M. and will be preceded by a cocktail party, the location of which will be announced later.

All members of the Association who plan to attend the Thirtieth Meeting are urged to make their reservations at once with the Palmer House, stating the time of arrival and how long they plan to remain in Chicago.

PROGRAM

FRIDAY, JUNE 18, 1948

8:30 A.M. Registration

I. 9:30 A.M. Red Lacquer Room

J. S. L. BROWNE, *presiding*

1. PSEUDO-GLANDULAR DISTURBANCES.
   by Hugo R. Rony
2. SYNDROME OF CYPTORCHIDISM, HEART DISEASE AND DERMATOSIS.
   by S. J. Glass
3. CONSTITUTIONAL PRECOCIOUS PUBERTY CONTROLLED BY ANDROGEN THERAPY.
   by S. Charles Freed and Minnie Goldberg
4. A STUDY OF THE BIOLOGICAL ACTIVITY AND THE MAGNITUDE OF ENDOGENOUS ANDROGEN PRODUCTION IN A CASE OF ANDRENOGENITAL SYNDROME.
   by Anne C. Carter and Ephraim Shorr
5. PSEUDOHERMAPHRODISM. EARLY AND LATE RECOGNITION.
   by M. James Whitelaw
6. CLINICAL, LABORATORY, OPERATIVE AND POSTMORTEM OBSERVATIONS IN IN-

FANTS AND CHILDREN WITH MULTIPLE CONGENITAL MALFORMATIONS (TURNER'S SYNDROME, OVARIAN AGENESIS AND RELATED COMBINATIONS).
by Frank L. Plachte (introduced by Henry H. Turner)

7. A SYNDROME CHARACTERIZED BY HYPERCALCEMIA. CALCINOSIS, AND RENAL INSUFFICIENCY FOLLOWING PROLONGED INTAKE OF CALCIUM AND ALKALI.
by Charles H. Burnett, Robert R. Commons (by invitation), Fuller Albright and John E. Howard

8. HYPOPARATHYROIDISM, WITH MENTAL TROUBLES AND ECTODERMAL DISORDERS.
by Manuel Villaverde

9. TREATMENT OF FAR ADVANCED INOPERABLE CARCINOMA OF THE BREAST WITH ESTROGENS AND ANDROGENS.
by Samuel G. Taylor, III, Danely Slaughter (by invitation) and Frederick W. Preston (by invitation)

10. HORMONAL FACTORS INVOLVED IN THE REGULATION OF BODY TEMPERATURE DURING MENSTRUAL CYCLE AND PREGNANCY.
by Charles L. Buxton and William B. Atkinson

11. THE EFFECTS OF CERTAIN STEROIDS—INTRAMUSCULAR AND SUBLINGUAL—ON THE BASAL BODY TEMPERATURE OF THE ADULT HUMAN MALE.
by Robert M. Perlman

II. 2:00 P.M. Red Lacquer Room

C. N. H. LONG, *presiding*

12. A SIMPLIFIED HYPOPHYSECTOMIZED RAT ADRENAL ASCORBIC ACID BIOASSAY METHOD FOR ADRENOCORTICOTROPHIN (A.C.T.H.); SPECIFICITY AND APPLICATION TO PREPARATIVE PROBLEMS.
by Paul L. Munson, Alfred G. Barry, Jr. (by invitation), and F. C. Koch

13. CONTENT OF ADRENOCORTICOTROPHIC HORMONE (A.C.T.H.) IN THE RAT PITUITARY UNDER OPTIMAL AND STRESSFUL ENVIRONMENTAL CONDITIONS.
by George Sayers, Marshal Merkin (by invitation) and J. N. Tortoreto (by invitation).

14. THE ACTIVATION OF THE ADRENAL CORTEX BY INSULIN HYPOGLYCEMIA.
by H. Gershberg (by invitation) and C. N. H. Long.

15. INFLUENCE OF ADRENOTROPHIC HORMONE ON SODIUM EXCRETION IN HYPOPHYSECTOMIZED RATS.
by Betty L. Rubin (by invitation) and Ralph I. Dorfman

16. FACTORS INFLUENCING THE CORTICOTROPHIN PRODUCTION OF THE ANTERIOR PITUITARY.
by Hans Selye

17. THE USE OF ANDRENOCORTICOTROPHIN AS A TEST OF ADRENAL CORTICAL RESERVE.
by George W. Thorn, Peter H. Forsham (by invitation), Lillian Recant (by invitation) and A. Gorman Hills (by invitation)

18. OBSERVATIONS ON THE PITUITARY-ADRENAL RESPONSE FOLLOWING EPINEPHRINE INFUSION IN MAN.
by Lillian Recant (by invitation), Peter H. Forsham (by invitation) and George W. Thorn

19. FATE AND METABOLIC ACTION OF INTRAVENOUSLY ADMINISTERED ADRENOCORTICOTROPHIC HORMONE (A.C.T.H.).
by Thomas W. Burns (by invitation), George Sayers, Frank H. Tyler (by

invitation), B. V. Jager (by invitation), T. B. Schwartz (by invitation), Emil L. Smith (by invitation) and L. T. Samuels

20. Metabolic Changes Following the Administration of Pituitary Adrenocorticotrophic Hormone (A.C.T.H.) to Normal Humans.
    by H. T. McAlpine (by invitation), E. H. Venning, L. Johnson (by invitation), V. Schenker (by invitation), M. M. Hoffman and J. S. L. Browne
21. The Effect of Adrenocorticotrophin on Antibody Levels in Normal Human Subjects.
    by P. H. Herbert and J. A. de Bries (introduced by J. S. L. Browne)
22. A Comparison of the Effect on Bone Formation of the Hyperadrenocorticism of Cushing's Syndrome with that Induced by Adrenocorticotrophic Hormone (A.C.T.H.).
    by Frederic C. Bartter (by invitation), Anne P. Forbes and Fuller Albright
23. Adrenal Cortical Unresponsiveness in Patients with Gastric Cancer.
    by Edward C. Reifenstein, Jr., N. F. Young (by invitation), Aurelia Potor (by invitation), Benedict Duffy (by invitation) and F. Homburger (by invitation)
24. The Excretion of Adrenal Metabolites in Human Urine.
    by Konrad Dobriner, Seymour Lieberman (by invitation) and C. P. Rhoads (by invitation)

III. Annual Dinner, Friday, June 18.

7:30 p.m.—Red Lacquer Room, Palmer House

Presentation of E. R. Squibb and Sons Award for 1948
Presentation of Ciba Award for 1948
Presentation of Ayerst, McKenna and Harrison Fellowship for 1948.
by Warren O. Nelson, Chairman of the Committee on Awards 1947–48

President's Address: C. N. H. Long, Yale University

Saturday, June 19, 1948

IV. 9:00 a.m. Red Lacquer Room

R. G. Hoskins, *presiding*

25. Preparation of Crystalline Growth Hormone.
    by Jacob B. Fishman (by invitation), Alfred E. Wilhelmi (by invitation) and Jane A. Russell
26. The Influence of Purified Growth Hormone on Fasting Metabolism.
    by Clara M. Szego and Abraham White
27. Unpredictable Effects of Growth Hormone Preparations on Nitrogen Storage.
    by Paul Bartlett (by invitation) and Oliver H. Gaebler
28. Studies in Growth. I. The Effects of Androgen in Gigantism and Acromegaly.
    by Laurence W. Kinsell, George D. Michaels (by invitation), Choh Hao Li (by invitation) and William E. Larsen (by invitation)
29. The Effect of Iodine Injections on Energy Metabolism and Plasma Protein-Bound Iodine of Rats.
    by S. B. Barker and H. J. Lipner (by invitation)

30. THE EFFECT OF PITUITARY AND NON-PITUITARY GLAND FACTORS ON THE FORMATION OF INTRACELLULAR COLLOID DROPLETS IN THE THYROID GLAND IN VIVO AND IN VITRO.
    by Samuel Dvoskin
31. INACTIVATION OF THE EXOPHTHALMIC, THYROTROPIC AND KETOGENIC PRINCIPLES OF ANTERIOR PITUITARY EXTRACT BY IODINATION.
    by William McK. Jefferies
32. NEWER METHODS OF ANTAGONIZING HYPERTHYROIDISM.
    by Robert H. Williams, Rene F. Tagnon (by invitation), Herbert Jaffe, (by invitation), Beverly T. Towery (by invitation) and Walter F. Rogers (by invitation)
33. THE USE OF RADIOACTIVE IODINE (I 131) IN THE STUDY OF NORMAL AND DISORDERED THYROID FUNCTION IN MAN.
    by Sidney C. Werner and Edith Quimby (by invitation)
34. THE EFFECT OF THYROID STIMULATING HORMONE ON THE FUNCTION OF HUMAN NORMAL AND MALIGNANT THYROID TISSUE.
    by J. B. Trunnell (by invitation), R. W. Rawson, L. D. Marinelli (by invitation) and Ruth Hill (by invitation)
35. THE RELATION BETWEEN INFANT BIRTHWEIGHT AND SUBSEQUENT DEVELOPMENT OF MATERNAL DIABETES MELLITUS.
    by Joseph P. Kriss and Palmer H. Futcher (introduced by Cyril M. MacBryde)

V. 2:00 P.M. Red Lacquer Room

A. T. KENYON, *presiding*

36. ABSORPTION AND EXCRETION OF CHORIONIC GONADOTROPHIN WHEN ADMINISTERED INTRAMUSCULARLY TO WOMEN.
    by J. T. Bradbury and Willis E. Brown
37. THE RENAL CLEARANCE OF CHORIONIC GONADOTROPHIC HORMONE IN PREGNANCY AND IN NEOPLASM OF THE TESTIS.
    by C. F. Gastineau (by invitation), A. Albert and L. M. Randall (by invitation)
38. THE METABOLIC RESPONSE TO CHORIONIC GONADOTROPHIN IN YOUNG MEN.
    by Kathryn Knowlton (by invitation) and Allan T. Kenyon
39. BLOOD GONADOTROPHIN STUDIES DURING PREGNANCY IN RELATION TO THE FETAL SEX.
    by H. E. Nieburgs and Robert B. Greenblatt
40. ON THE PRINCIPAL ESTROGENIC CONSTITUENTS OF THE URINE OF THE STALLION.
    by Louis Levin
41. MECHANISM OF INACTIVATION OF α-ESTRADIOL BY RAT LIVER IN VITRO.
    by R. H. deMeio (by invitation), A. E. Rakoff, A. Cantarow and K. E. Paschkis
42. COZYMASE IN THE HEPATIC INACTIVATION OF α-ESTRADIOL.
    by Richard L. Coppedge (by invitation), Albert Segaloff, Herbert Sarett (by invitation) and Aaron Altshul (by invitation)
43. INTERFERENCE WITH ESTROGEN-INDUCED GROWTH IN THE FEMALE GENITAL TRACT BY FOLIC ACID.
    by Roy Hertz
44. THE RELATION OF FOLIC ACID TO THE ACTION OF ESTROGENS.
    by Irene T. Kline (by invitation) and Ralph I. Dorfman

45. Fluorescent Phenomena of the Vulva Associated with Sex Hormone Metabolism.
 by M. Sydney Margolese
46. Testicular Deficiency: A Clinical and Pathological Study.
 by R. Palmer Howard, Ronald C. Sniffen (by invitation) and Fred A. Simmons
47. A Comparison of the Effect of Various Androgens on the Temporal Muscle and Organs of the Castrated Male Guinea Pig.
 by Charles D. Kochakian and Jane Harrison Humm (by invitation)

VI. Annual Business Meeting

5:00 p.m. Red Lacquer Room

Papers to be Read by Title

48. The Use of Whole Adrenal Cortical Extract in Experimental Infections.
 by Erwin P. Vollmer, James D. Gillmore (by invitation), Leo Cravitz (by invitation) and J. E. Samsell (by invitation).
49. The Work Performance of Adrenalectomized Rats Given Continuous Intravenous Infusions of Glucose.
 by Dwight J. Ingle and James E. Nezamis (by invitation)
50. Sublingual Administration of Desoxycorticosterone Acetate in the Treament of Addison's Disease.
 by Evelyn Anderson, Lawrence W. Kinsell, Troy C. Daniels (by invitation) and Edward Henderson
51. Excretion of Adrenal Metabolites Following the Administration of Adrenocorticotrophic Hormone to Normal Human Subjects.
 by Eleanor H. Venning, V. E. Kazmin (by invitation), Miriam Ripstein (by invitation), H. T. McAlpine (by invitation) and M. M. Hoffman
52. The Effect of 11-Dehydrocorticosterone on Fecal Fat Excretion.
 by Grace E. Bergner (by invitation), Roger A. Lewis (by invitation), Frances W. Stout (by invitation), George W. Thorn and Kendall Emerson, Jr.
53. An Estimation of the Quantity of 11-17-Oxysteroid Excretion by the Human Adrenal Stimulated by ACTH.
 by A. Gorman Hills (by invitation) and George W. Thorn
54. Isolation of Urinary Steroids from a Patient with Apparent Adrenal Involvement.
 by A. M. Miller (by invitation) and Ralph I. Dorfman
55. The Effect of Adrenalectomy and Desoxycorticosterone Acetate Administration upon the ECG Response of the Rat to Cardiac Glycosides.
 by Herbert S. Kupperman, Joseph G. Benton (by invitation) and Arthur C. DeGraff (by invitation)
56. Activation of the Adrenal Cortex in Human Subjects Following Electroconvulsive Therapy (E.C.T.) and Psychomotor Stress.
 by R. A. Cleghorn and A. J. Goodman (by invitation), B. F. Graham, M. H. Jones and N. K. Rublee
57. Effect of Adrenal Cortical Compounds on Electrolyte Metabolism

of a Patient with Addison's Disease During High Sodium Chloride Intake.
by Aurelia Potor (by invitation), Nelson F. Young (by invitation), F. Homburger (by invitation) and Edward C. Reifenstein, Jr.

58. Nitrogensparing (Protein-Anabolic) Action of Thyroid Hormone.
by J. Rupp (by invitation) and K. E. Paschkis

59. Thiouracil Effect on Plasma and Liver Protein Concentrations.
by James H. Leathem

60. Radioiodine Uptake by the Thyroid as an Aid in Differential Diagnosis.
by S. M. Seidlin, E. Oshry (by invitation), I. Rossman (by invitation) and L. Leiter (by invitation)

61. Thyroid Uptake of Radioactive Iodine in the Normal and Hypometabolic Human.
by Martin Perlmutter (by invitation) and Peter H. Forsham (by invitation)

62. Cellular Involution in the Thyroid.
by Nathan B. Friedman

63. Methyl Thiouracil in the Treatment of Thyrotoxicosis.
by Grosvenor W. Bissell, John M. Benny (by invitation), Victor Totah (by invitation) and Florence Gilbert (by invitation)

64. Modification of the Estrual Cycle of the Ewe by the Use of Progesterone; the Effect Upon Subsequent Ovulation Rate and Fertility of Ova.
by R. H. Dutt (by invitation) and L. E. Casida

65. Effects of Various Estrogenic Preparations on the Vaginal Mucosa.
by Mildred Vogel (by invitation), Thomas H. McGavack and Joseph Mellow (by invitation)

66. The Use of the Vaginal Smear in the Assay of Estrogens Given Orally or Intramuscularly.
by Willis E. Brown and J. T. Bradbury

67. The Similarity of Estrogenic Effect in Premenstrual Tension, Menstrual Anomalies, Chronic Cystic Mastitis and Cancer of the Breast.
by Joseph H. Morton

68. Hyperestrogenism Treated with Lactogenic Hormone (Prolactin).
by Manuel Villaverde

69. The Factor of Rhythm in Experimental Menstruation.
by Doris H. Phelps

70. Hormonal Pellets in the Management of the Menopausal Syndrome.
by Robert B. Greenblatt and Roland R. Suran (by invitation)

71. The Effect of Hypophysectomy on the Ovulability of the Ovarian Follicle of the Domestic Hen.
by Irving Rothchild and R. M. Fraps

72. Prognostic Value of Pregnanediol Excretion in Threatened Abortion with Special Reference to the Effects of Diethylstilbestrol.
by A. R. Abarbanel

73. Further Studies on the Endometrial Cups of the Pregnant Mare.
by H. H. Cole and G. H. Hart

74. Inactivation of Posterior Pituitary Antidiuretic Hormone of the Liver.
by W. J. Eversole, J. H. Birnie (by invitation) and Robert Gaunt

75. The Estimation of Dehydroisoandrosterone and Related Compounds

ANNOUNCEMENT OF THE 1948 LAURENTIAN HORMONE CONFERENCE

The Laurentian Hormone Conference of the A.A.A.S. will meet in 1948 at the Forest Hills Hotel, Franconia, New Hampshire, from September 13th to 18th inclusive. The program (which will be published in full at a later date) will consist of four sections:

I. The metabolism of steroid hormones *in vivo* and *in vitro*.
II. Thyroid physiology and function.
III. The role of hormones in tissue and body metabolism.
IV. Hypothalamic neuro-humoral relationships.

Because of limited accommodations, attendance is by invitation, but the Committee on Arrangements will receive applications for membership until June 15, 1948. Applications should be addressed to: Dr. Gregory Pincus, Chairman, Committee on Arrangements, 222 Maple Avenue, Shrewsbury, Massachusetts.

The Journal of CLINICAL ENDOCRINOLOGY

VOLUME 8 MAY, 1948 NUMBER 5

CLASSIFICATION OF MALE HYPOGONADISM AND A DISCUSSION OF THE PATHOLOGIC PHYSIOLOGY, DIAGNOSIS AND TREATMENT*

CARL G. HELLER, M.D., PH.D.† AND WARREN O. NELSON, PH.D.‡

From the University of Oregon Medical School, Portland, Oregon, and the University of Iowa College of Medicine, Iowa City, Iowa

INTRODUCTION

CLASSIFICATION, diagnosis and treatment of testicular failure have been radically altered and at the same time have been placed on a rational basis by the introduction of two important technics: 1) determining the alterations in microscopic anatomy of the testis from a study of biopsy specimens, and 2) determining the urinary excretion of pituitary gonadotropic hormones. The biopsy studies reveal whether the testicular

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* Read in part before the annual meeting of the Central Society for Clinical Research, Chicago, Illinois, November, 1942 (Abst. *J.A.M.A.* 121: 1176, 1943) and in part before a meeting of the North Pacific Society of Internal Medicine, Portland, Oregon, October 27, 1945.

The patients comprising the basis of this dissertation were studied at Wayne University College of Medicine and the City of Detroit Receiving Hospital, Detroit, and the Multnomah County Hospital and the University of Oregon Medical School, Portland.

The study was supported in part by a grant from the American College of Physicians, and in part by a grant from the Schering Corporation, Bloomfield, New Jersey, through the courtesy of Dr. Edward Henderson.

† Associate Professor of Physiology and Medicine.

‡ Professor of Anatomy.

lesion involves primarily the seminiferous tubules or the interstitial cells of Leydig. Biopsy studies also indicate the prognosis and treatment insofar as they reveal whether the testicular tissue is irreparably damaged or is potentially capable of being stimulated. The urinary gonadotropins reveal whether the testicular failure is secondary to pituitary failure or whether it is primary. Thus instances of gonadal failure may be divided into two distinct groups: 1) those having distinctly higher than normal titers of gonadotropins—the *hypergonadotropic syndromes*, and 2) those having distinctly subnormal titers of gonadotropins—the *hypogonadotropic syndromes*.

In classifying the true hypogonadal states it is necessary to know whether the deficiency developed after maturity was reached or whether it was present early enough in life to prevent the patient from undergoing puberty. This knowledge permits the separation of cases into two large groups, each of which can be identically subdivided into four physiologic categories, dependent upon the primary site of failure. Thus in each group the hypogonadism may be due to 1) failure of all the functions of the anterior pituitary, 2) failure of the pituitary to elaborate gonadotropic hormone, 3) failure of the interstitial cells of Leydig of the testis, and 4) failure of the seminiferous tubules of the testis.

Failure at each of the four sites will lead to failure of spermatogenesis.

1) When the entire pituitary gland is affected—for example, by atrophy—neither the gonadotropic complex nor the other tropic hormones will be elaborated and consequently the Leydig cells and seminiferous tubules will fail to function. This can be readily understood by referring to the diagram of the physiologic control of the testis (Fig. 1).

2) If only the gonadotropic hormones fail to be secreted by the pituitary, this too will lead to Leydig cell and seminiferous tubule failure.

3) Primary failure of the interstitial cells of Leydig, which normally elaborate androgens, may result in abeyance of spermatogenesis since androgens maintain the seminiferous tubules as well as the rest of the genital tract in a functional state (1).

4) Failure on the part of the seminiferous tubules alone leads to failure of spermatogenesis without signs of hormonal deficiency.

Patients with any of the first three types of hormonal failure will have the usual clinical manifestations of androgen deficiency. If the onset of such failure antedates puberty, the condition will be characterized by changes in the bony skeleton; namely, dwarfism in the case of panhypopituitarism, and eunuchoidism in the other two instances. If the failure occurs after the individual has matured, no skeletal changes and often very little change in secondary sex characteristics will be noted. On clinical grounds alone it is often impossible to determine whether the hypogonadism is the result of primary testicular failure or of primary pituitary fail-

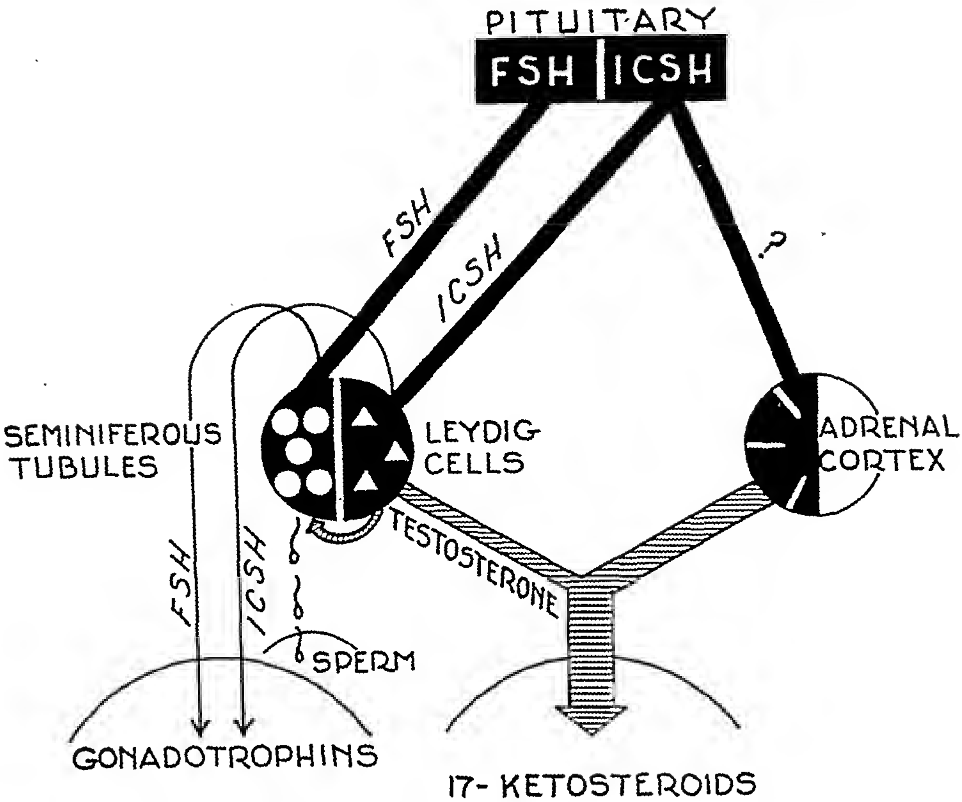

FIG. 1. PHYSIOLOGIC CONTROL OF THE TESTIS

F.S.H. refers to the follicle-stimulating hormone, which has a gametogenic function in males and females. It stimulates the germinal elements of the testis, promoting spermatogenesis in the seminiferous tubules (represented by circles). Note that testosterone plays a role in the maintenance of the seminiferous tubules.

I.C.S.H. refers to the interstitial-cell-stimulating hormone which stimulates the Leydig cells (represented by triangles) to produce testosterone. Formerly I.C.S.H. was known as luteinizing hormone (L.H.).

Together these two fractions constitute the *gonadotropic fraction*. In human urine the resultant action of F.S.H. plus I.C.S.H. is determined biologically.

17-Ketosteroids are hormones or their metabolites found in urine; approximately $\frac{3}{8}$ is derived from the testes and $\frac{5}{8}$ is derived from the adrenals. The term "17-keto" refers to the ketone (=0) attached to carbon atom number 17 of the steroid molecule.

Normal sperm count, morphology and motility indicate the entire system is functionally intact.

ure leading to testicular failure. Since the type and length of treatment depend upon this differentiation, it is important to distinguish between them.

Differentiation can be accomplished by either of two means: 1) favorable response to a simple therapeutic test employing injections of chorionic gonadotropic hormone (*see* next section); or 2) by determining the urinary

TABLE 1. CLASSIFICATION OF MALE HYPOGONADISM

Time of onset of hypogonadism (from patient's history and physical examination)	A. Gonadotropin titer determined by urinary assay B. *Response to chorionic gonadotropin*	Failure of other pituitary tropic hormones	Skeletal development	Gynecomastia	Testicular development, grossly. (++++ normal)	Testicular biopsy (microscopic)	Sexual potency	Definitive diagnosis
Before or during puberty	A. Low	Yes	Dwarfism	None	++	Infantile tubules and Leydig cells	Poor	(1) *Panhypopituitary dwarfism*
	B. *Positive response*	No	Eunuchoidal	None	++	Infantile tubules and Leydig cells	Poor	(2) *Hypogonadotropic eunuchoidism*
	A. High	No	Eunuchoidal	None to marked	0	Absent or very atrophic testes (Wolffian duct derivatives in scrotum)	Poor	(3) *Prepuberal noncastrate eunuchism*
	B. *Negative response*	No	Normal or eunuchoidal	None to marked	+	Hyalinized tubules and clumping of Leydig cells	Normal	(4) *Puberal seminiferous tubule failure* (Klinefelter syndrome)
After attaining sexual maturity	A. Low	Yes	Normal	None	+++	Tubules and Leydig cells returned to infantile state	Poor	(1a) *Adult panhypopituitarism* (Simmonds' cachexia)
	B. *Positive response*	No	Normal	None	+++	Tubules and Leydig cells returned to infantile state	Poor	(2a) *Adult hypogonadotropic hypogonadism*
	A. High	No	Normal	None	++++	Absence, disorganization or atrophy of tubules and Leydig cells	Poor	(3a) *Adult hypergonadotropic hypogonadism* (male climacteric, or surgical castration)
	B. *Negative response*	No	Normal	None	+++ to ++++	Tubules reduced in size or hyalinized. Sloughing of germ cells. Leydig cells normal	Normal	(4a) *Adult seminiferous tubule failure* (most instances of irreversible sterility)

output of the gonadotropic hormones (2, 3). In primary testicular failure the output of pituitary gonadotropins is several times higher than in urines of normal men. When pituitary failure is responsible for the hypogonadism, urinary gonadotropins are either absent or well below the range of normal.

Another aid in distinguishing the four types of adult and prepuberal failure is a study of the variations in the microscopic anatomy of testicular biopsies.

By using each of the means mentioned, the eight distinct syndromes can be differentiated. This "key" to the hypogonadal states, as outlined in Table 1, may be applied to an individual patient in whom the tentative diagnosis is "hypogonadism." The definitive diagnosis can then be made. It also serves as a preview of the discussion to follow.

Therapeutic test with chorionic gonadotropin

This therapeutic test constitutes a pivotal point in the classification as well as in subsequent management of the patient suffering from hypogonadism. Favorable response to injection of chorionic gonadotropin as judged by growth of the genitalia signifies that the patient is lacking in gonadotropins since the patients with hypergonadotropic hypogonadism will not respond. As is amplified later, patients that respond may frequently be completely restored to normal by a course of chorionic gonadotropin therapy. In distinct contrast, the injudicious use of testosterone may cause sufficient testicular and pituitary suppression to preclude the possibility of subsequent stimulation of the testis. This undesirable possibility emphasizes the importance of proper classification and treatment.

The therapeutic test consists of the administration of 750 international units of chorionic gonadotropin intramuscularly *twice* daily for three weeks. If a response is elicited, the patient has hypogonadotropic hypogonadism and should be treated as outlined under this category. If no response is elicited and the patient is definitely hypogonadal, it can be safely assumed that the patient belongs to the hypergonadotropic category and that the primary site of involvement is the testis. Such a patient may be given substitutional therapy with testosterone, as outlined for the group of prepuberal noncastrated eunuchs.

The subsequent response of the patient bears out the findings obtained by the therapeutic test with chorionic gonadotropin. By actual assay of urinary gonadotropins a direct correlation is established. (In 100 per cent of the patients that responded favorably to the test, low urinary gonadotropic titers were found in the preliminary control period.) Additional confirmation is obtained from the microscopic anatomy of the testicular biopsies which show failure of stimulation of the Leydig cells and tubules, presumably because of the lack of gonadotropins. Conversely a positive corre-

lation is established between failure to respond to the test, high titers of urinary gonadotropins and biopsy evidence that the testes are absent or in such a state of damage that response to stimulation cannot be expected.

Classification into syndromes will follow the numbering system used in the key.

THE HYPOGONADOTROPIC SYNDROMES

FAILURE OF ALL FUNCTIONS OF THE ANTERIOR PITUITARY

1) **Panhypopituitary dwarfism.** Although this syndrome is rare, it is easily recognized by the short stature associated with lack of pituitary stimulation (a) to the thyroid gland, leading to hypothyroidism or myxedema; (b) to the gonads, leading to characteristic sexual infantilism; and (c) to the adrenal cortex, leading to an Addisonian-like picture.

1a) **Adult panhypopituitarism** (Simmonds' cachexia) is similarly rare and easily recognized by the failure of the thyroid, gonads and adrenals. It is often indistinguishable from the functional type of panhypopituitarism (anorexia nervosa) in both its clinical and laboratory manifestations.

A therapeutic test with chorionic gonadotropin elicits a positive response in both the prepuberal and adult types. Further, the urinary gonadotropin titers are low or absent, and testicular biopsies indicate the lack of pituitary stimulation. Evidence of insufficiency of the other tropic hormones distinguishes panhypopituitarism from the other hypogonadotropic syndromes.

Ideal therapy would consist of administering the various tropic fractions which are lacking, for example, growth, thyrotropic, adrenocorticotropic, follicle-stimulating, and interstitial-cell-stimulating hormones. Since these are not yet commercially available, administration of desiccated thyroid, adrenal cortical extract and the sex hormones is often resorted to with variable success.

FAILURE OF PITUITARY GONADOTROPIC SECRETION ONLY

2) **Hypogonadotropic eunuchoidism** superficially resembles other forms of hypogonadism in which the individual fails to undergo puberty. It is distinguished from panhypopituitarism in that the individual is tall rather than dwarfed and there is no evidence of deficiency of other pituitary hormones. The clinical features shared with other types of eunuchoidism (the prepuberal noncastrated type and the eunuchoidal type of seminiferous tubule failure) are: tall eunuchoidal skeletal structure (the span is two or more inches greater than the height, and the measurement from soles to pubic symphysis is one or more inches greater than the measurement from pubic symphysis to the vertex), delay in epiphyseal closure, poor development of paranasal sinuses and larynx, high pitched voice, subnormal de-

velopment of the male hair pattern, lack of recession of scalp hair line, poor muscular development and endurance, and sexual infantilism.

The following clinical features help to delineate hypogonadotropic eunuchoidism (Fig. 2) from other types of eunuchoidism: (a) more "progeria-like" wrinkling of the skin of the face; (b) although pubic, axillary

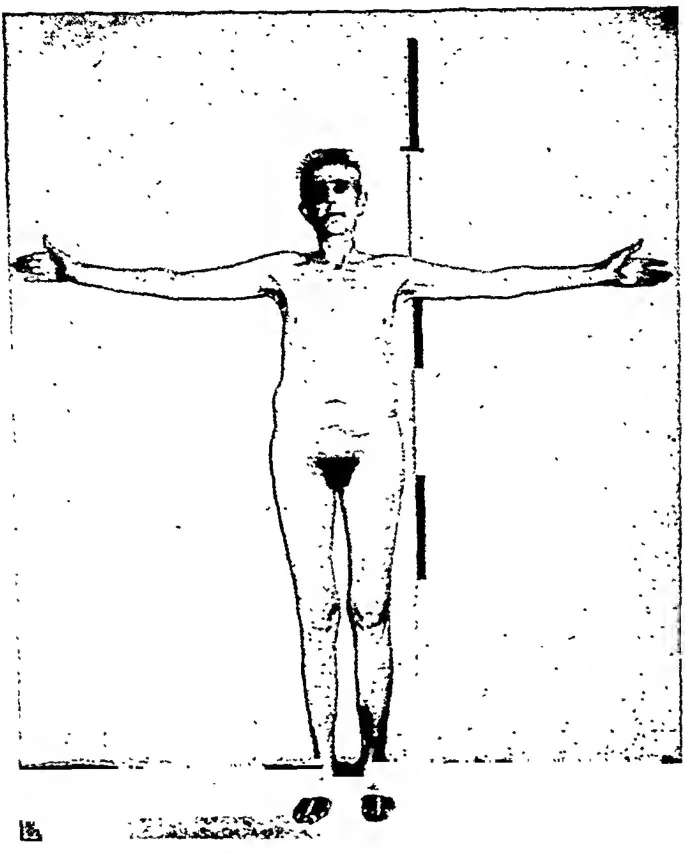

FIG. 2. Hypogonadotropic eunuchoidism in an untreated patient 51 years of age. Height, 70.5 in.; span, 73.3 in.; soles to pubic symphysis, 39.5 in.; pubic symphysis to vertex, 31 in. Roentgenograms revealed hyperostosis frontalis interna.

and extremity hair is decidedly less than in sexually normal males, more is present than in prepuberal noncastrated eunuchs. This may be correlated with (c) the level of excretion of 17-ketosteroids, which falls at or below the lower limits of normal but is higher than in the noncastrated eunuch. (d) Testes are always present but are smaller than normal, whereas the noncastrated eunuchs have none and patients with seminiferous tubule failure have very firm atrophic testes. (e) Mammary glands remain unde-

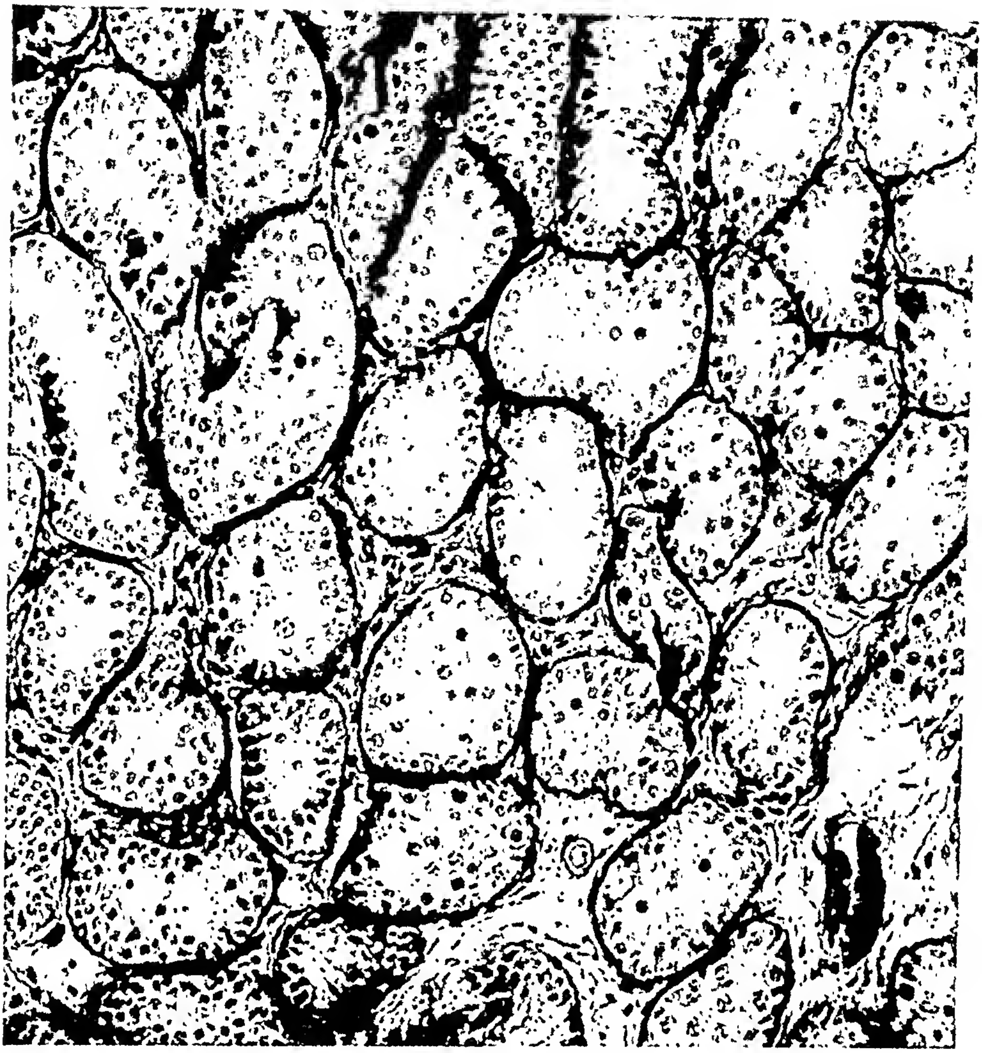

FIG. 3. Testicular biopsy (×175) from a patient 26 years of age with untreated hypogonadotropic eunuchoidism. Note presence of mesenchymal cells and absence of Leydig cells in the interstitial spaces, the small caliber of the seminiferous tubules and the absence of spermatogenesis beyond the spermatocyte stage. This biopsy reveals a suitable substrate, potentially capable of being stimulated.

veloped as in normal males, whereas in the other two syndromes there may be either gross or microscopic (biopsy) evidence of mammary stimulation.

The diagnosis is suggested by the presence of the findings described above, and established by:

1. A positive response to the administration of chorionic gonadotropin in adequate amounts.
2. Urinary gonadotropin titers below normal (or absent).
3. Testicular biopsies, revealing the presence of Leydig cells and some elements of the germinal series in an undeveloped state. These biopsies (Fig. 3) resemble testes seen in prepuberal boys and are somewhat similar to those of hypophysectomized animals.

Therapeutic program suggested for treatment of hypogonadotropic eunuchoidism: It is evident from the gonadotropin titers and testicular biopsies that the physiologic defect responsible for this syndrome is a lack of both interstitial-cell-stimulating hormone (I.C.S.H.) and follicle-stimulating hormone (F.S.H.). This suggests the use of these two hormones in any therapeutic program designed to stimulate the testes.

A. Administration of chorionic gonadotropin. In order to correct the hormonal deficiency of the testes a potent I.C.S.H. preparation capable of stimulating the Leydig cells is necessary. No purified I.C.S.H. of anterior pituitary origin is commercially available. Preparations of gonadotropin from the serum of pregnant mares and of chorionic gonadotropin from the urine from pregnant women, both have potent I.C.S.H. effects. However, the former has predominantly an F.S.H. effect whereas purified preparations of chorionic gonadotropin are regarded as free of F.S.H. effect. Since we have been interested in elucidating mechanisms of action throughout our studies we have elected to use those preparations which have the activity of a single hormone. In this instance chorionic gonadotropin was the substance chosen. Synonyms for this substance are: prolan, pregnancy urine extract, placental gonadotropin and anterior pituitary-like hormone. Chorionic gonadotropic hormone is presumably secreted by the chorionic cells of human placenta and is derived commercially from the urine of pregnant women. It is a complex protein substance with an unknown chemical formula and it has not been synthesized. It is standardized in international units.

The commercial preparations, currently available on the market, which were used in this study include: "Pranturon" (Schering), "Korotrin" (Winthrop), "Chorionic gonadotropin" (Upjohn), "A.P.L." (Ayerst, McKenna and Harrison), and "Antuitrin-S" (Parke, Davis & Co.).[1] All proved to be equally effective when employed under similar conditions. Others currently available include "Follutein" (Squibb), "Chorionic gonadotropin" (Armour), "Chorionic gonadotropin " (Sharpe and Dohme), and "Pregnyl" (Roche-Organon).

In our experience the most efficient mode of administration is as follows: inject 750 international units intramuscularly twice daily for at least four to six weeks. The dose may then be reduced by one-half and continued an additional two months. Allow a rest period of from three to six months. At the end of this period it must be ascertained whether the developmental changes effected by the homone have been maintained or have regressed,

[1] We are especially grateful to Dr. Gifford Upjohn of the Upjohn Company, Kalamazoo, Michigan, for providing us with well over one million units, and Dr. Edward Henderson of the Schering Corporation, Mr. Carr of Winthrop, and Dr. Murray Scott of Ayerst, McKenna and Harrison for the generous supplies of chorionic gonadotropin they provided.

or whether there has been further development subsequent to the cessation of therapy. The last situation indicates spontaneous improvement and in this event improvement may continue without any further treatment. In

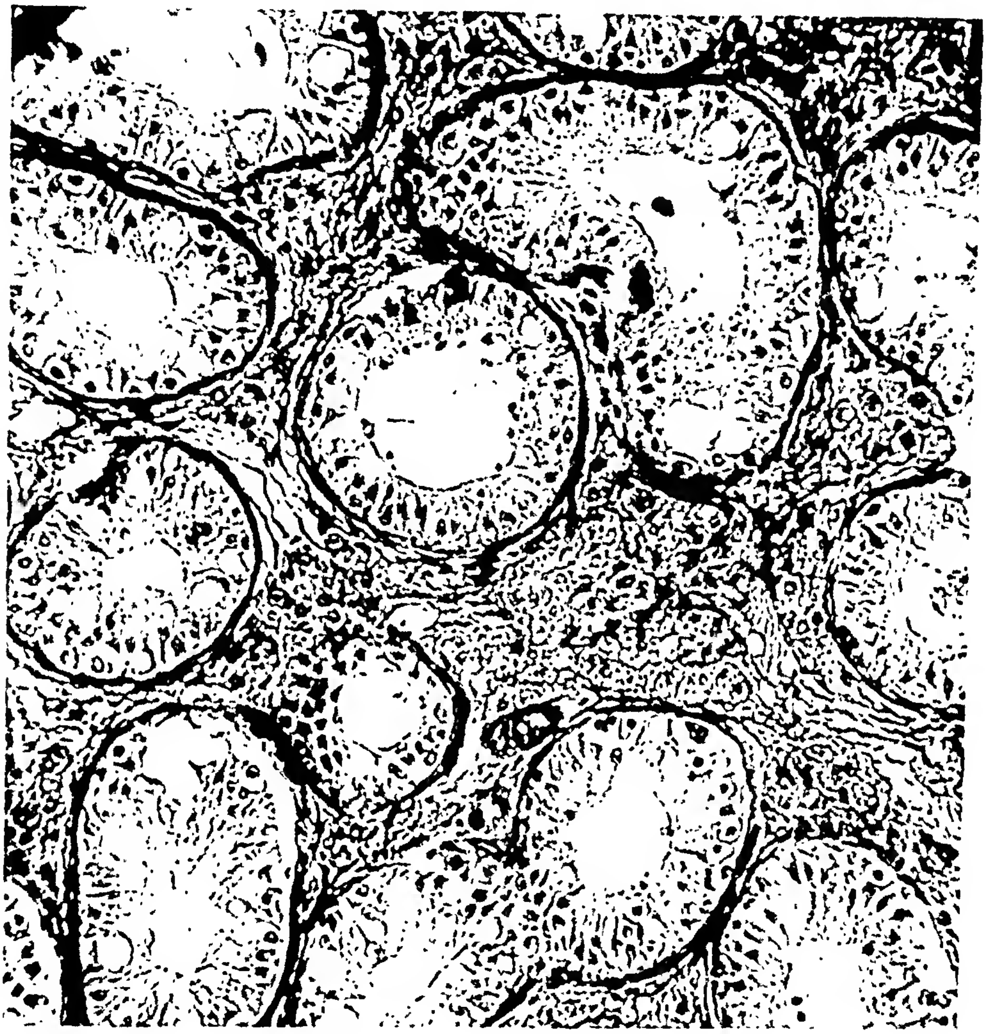

FIG. 4. Testicular biopsy (×175). A case of hypogonadotropic eunuchoidism after treatment with chorionic gonadotropin in a dose of 1500 I.U. 5 times weekly for four months. Note appearance of Leydig cells in the interstitial spaces. These were producing androgen, as may be seen from Figure 5, which shows the genitalia before and after the four-month period. Note larger tubules (due to androgen stimulation) and lack of spermatogenesis beyond spermatocyte stage.

the other instances further therapy is necessary. The usual procedure is to alternate three-month periods of treatment with three-month periods of rest. A surprisingly large proportion of the cases of hypogonadotropic eunuchoidism will maintain their improved status or actually continue to develop toward normal without further therapy after six to eighteen

months on this program. One plausible explanation is that a trigger mechanism, which had failed to be set off at the time of the normally expected puberty, has been set off by the administration of chorionic gonadotropin. Thus the previously underactive pituitary gland, in some unexplained manner, has been stimulated to secrete gonadotropins. The process, having once been initiated, continues spontaneously.

Testicular biopsies taken on the same individual before and after

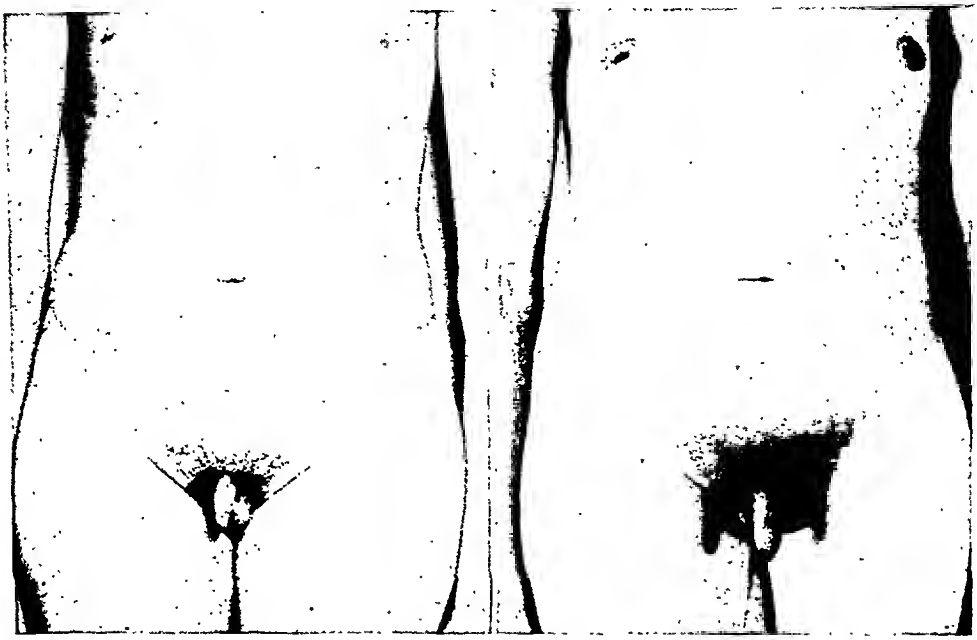

FIG. 5. Hypogonadotropic eunuchoidism in a man 26 years of age, before and after four months of chorionic gonadotropin therapy. The testicular biopsies shown in Figures 4 and 6 are from this patient.

chorionic gonadotropic therapy show marked stimulation of the Leydig cells and increase in size of the seminiferous tubules but no increase in spermatogenesis (Figs. 3 and 4). This indicates that chorionic gonadotropic hormone stimulates Leydig cell growth and secretion.

This therapy has been uniformly successful in all cases. This success can be ascribed to three important factors: 1) establishment of the proper pathologic-physiologic diagnosis; 2) use of adequate amounts of the hormone; and 3) administration of it at frequent intervals. The use of large amounts of hormone at frequent intervals seems complicated and expensive. The gratifying results accomplished over a short period (Fig. 5) and the

lack of necessity for continued treatment in most cases, actually makes this method less expensive than the use of inadequate doses at infrequent intervals over longer periods of time.

FIG. 6. Testicular biopsy (×175). The same case of hypogonadotropic eunuchoidism shown in Figures 4 and 5, after adding follicle-stimulating hormone to the therapy (one-half cc. of F.S.H., 519, prepared by Dr. H. McShan of the University of Wisconsin. was administered intramuscularly 5 times weekly for three months, omitted for six weeks, then administered again in a dose of 1 cc. 5 times weekly for two months, at which time spermatozoa were noted in the seminal fluid for the first time and a biopsy specimen was procured). Note that all stages of spermatogenesis are present and the tubules are larger than in Figure 4.

B. Administration of follicle-stimulating hormone. Chorionic gonadotropic hormone therapy corrects only the hormonal but not the germinal deficiency of the hypogonadotropic eunuchoid. We have been fortunate in having a purified follicle-stimulating hormone prepared by and made

available through the courtesy of Dr. H. McShan of the University of Wisconsin, which has been added to the therapeutic regimen of some patients following their response to chorionic gonadotropin. Testicular biopsies taken before adding F.S.H. revealed no spermatogenesis, and the seminal fluid contained no spermatozoa. F.S.H. was given once daily intramuscularly in large doses and biopsies were obtained from two to three months later. Testicular biopsies (Fig. 6) and examination of seminal fluid both indicated that F.S.H. administration had stimulated spermatogenesis.

Unfortunately, so far as we know, no purified or highly potent F.S.H. is as yet available commercially. However, there are mixtures of F.S.H. and I.C.S.H. derived from anterior pituitary sources on the market which may prove to be of benefit. We have employed "Preloban Niphanoid" (Winthrop) with some success. Since no uniform standards are available for the mixture no specific recommendations as to dosage can be made.

2a) **Adult hypogonadotropic hypogonadism.** No skeletal changes are to be expected and, in general, masculine characteristics are retained. Our single example of this condition exhibited slight regression of facial and bodily hair growth, sexual impotence, and symptoms associated with the male climacteric. Administration of chorionic gonadotropin reversed the symptom complex.

THE HYPERGONADOTROPIC SYNDROMES

FAILURE OF LEYDIG-CELL FUNCTION

In this type of primary testicular failure, as well as in the syndromes where the seminiferous tubules are mainly involved, the chorionic gonadotropins elicit no response, titers of urinary gonadotropins are higher than normal, and biopsies fail to reveal testicular tissue capable of stimulation. The four syndromes to be discussed here as examples of primary testicular failure are therefore also to be called the *hypergonadotropic syndromes*.

3) **Prepuberal noncastrated eunuchs**[2] (Fig. 7) are eunuchoidal individuals who either failed to develop testes (testicular agenesis) or whose testes underwent complete atrophy before the age of puberty was reached (5). Surgical castrates whose orchidectomy antedated puberty may be included, since, according to Wagenseil (6), they have the same clinical features and similarly elevated gonadotropin titers (7).

In addition to having the features common to all types of eunuchoidism (listed in part under hypogonadotropic eunuchoidism) the prepuberal noncastrated eunuchs have the following distinguishing features: (a) diminution of scrotal contents, often leading to the incorrect diagnosis of

[2] Also referred to as functional prepuberal castrates.

abdominal testes; (b) the least pubic and axillary hair encountered in any of the eunuchoidal syndromes; (c) very low 17-ketosteroid excretion; (d) gynecomastia which is usually obvious on physical examination but occasionally discovered only by a mammary biopsy; (e) no response to the administration of chorionic gonadotropins; (f) high titers of urinary gonadotropins. Biopsies of the scrotal contents reveal the presence of

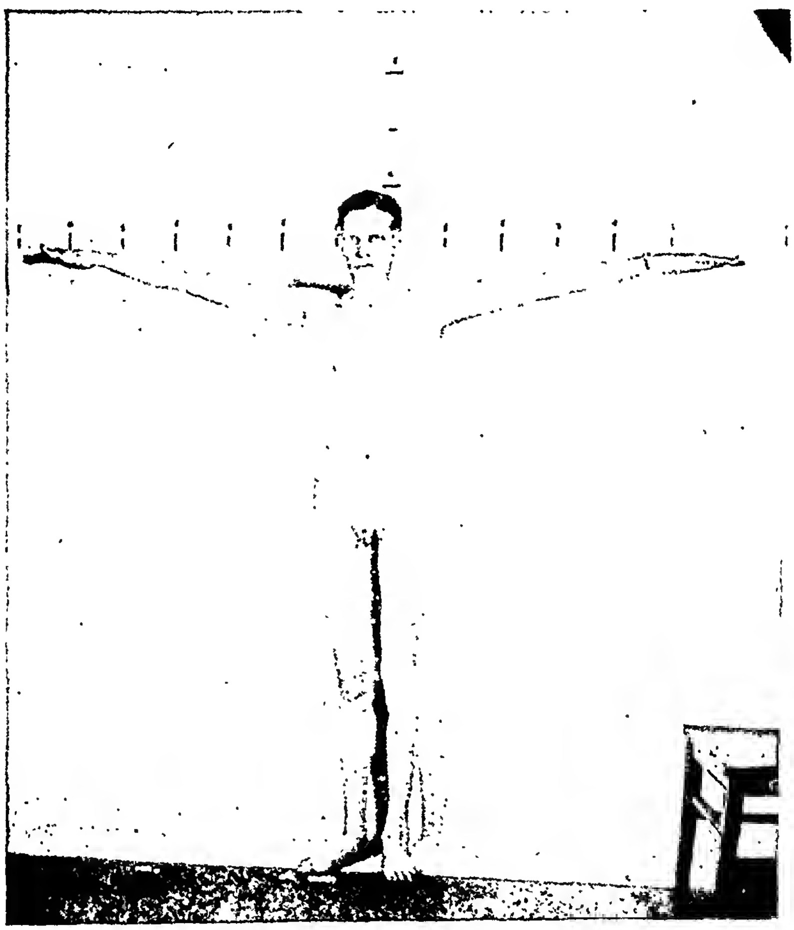

FIG. 7. Prepuberal noncastrated eunuch 32 years of age.

Wolffian duct derivatives, but absence of functional testes (Fig. 8). Remnants of atrophic testes are occasionally discovered.

Therapy must necessarily be substitutional in nature. Twenty-five mg. of testosterone propionate[3] administered five times weekly intramuscularly is satisfactory. Implantation of six to eight pellets of testosterone weighing

[3] Testosterone propionate (Oreton) and testosterone pellets were kindly provided by the Schering Corporation, Bloomfield, New Jersey, through the courtesy of Mr. George Straayer and Dr. Edward Henderson.

75 mg. each is more convenient and gives equivalent results. Implantation needs to be repeated only every six or eight months.

3a) **Adult hypergonadotropic hypogonadism.** As in other instances where gonadal failure develops after maturity is reached, the normal male habitus is largely retained. Only after years of testicular deficiency does one note any regression of hair growth or diminution in genital size. The chief complaints of this group of patients are either sexual impotence or symptoms

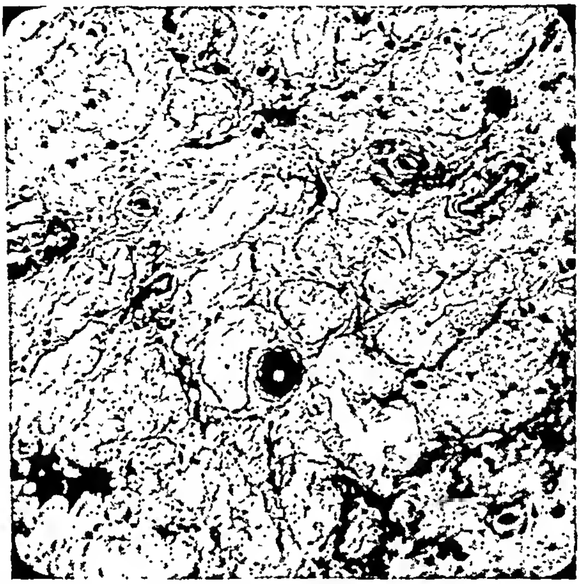

FIG. 8. Biopsy of scrotal contents (X175) in a prepuberal noncastrated eunuch 52 years of age. Note remnants of seminiferous tubules. This biopsy fails to reveal a substrate capable of being stimulated (reproduced from Heller, C. G.; Nelson, W. O., and Roth, A. A.: *J. Clin. Endocrinol.* 3: 573, 1943).

of the male climacteric (8). It is interesting to note that climacteric symptoms arise only in individuals who at one time have had a fair degree of Leydig cell function, suggesting that the symptoms are related to withdrawal of the sex hormones. Climacteric symptoms may arise as a consequence of gonadal failure secondary to pituitary failure as well.

To substantiate the diagnosis of adult hypergonadotropic hypogonadism as the factor responsible for impotence or climacteric symptoms, one needs to find (a) high titers of urinary gonadotropins, or (b) evidence of failing Leydig cell function in a biopsy specimen, or (c) lack of response to chorionic gonadotropin plus a definite response to testosterone (8) administered in adequate amounts such as 25 mg. five to seven times weekly intramuscu-

larly for two weeks. A positive response indicates that testicular failure is present whereas a negative response suggests psychogenic impotence or psychoneurosis. In some instances the etiology of the testicular failure is apparent, for example, bilateral orchitis, trauma, surgical castration, or interference with testicular blood supply following herniorrhaphy, In most instances, however, no etiologic factor can be elicited.

Treatment is as outlined for prepuberal noncastrated eunuchs.

SEMINIFEROUS TUBULE FAILURE

4) **Puberal seminiferous tubule failure** (9) is the most common form of gonadal failure and yet is least often recognized. The primary defect con-

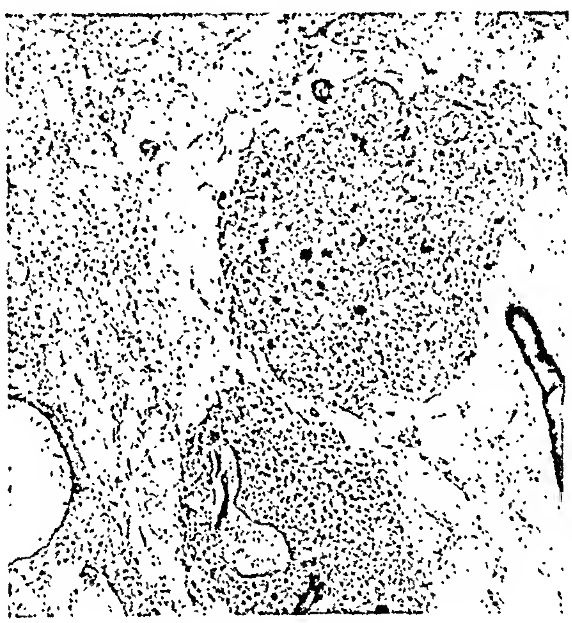

Fig. 9. Testicular biopsy (×80). Puberal seminiferous tubule failure. Note hyalinized seminiferous tubules, occasional tubule devoid of cells of the germinal series and containing Sertoli cells only, and aggregation of Leydig cells of small type.

sists of disappearance of germinal cells and hyalinization of the seminiferous tubules, but this rarely exists without concomitant involvement of the Leydig cells although to a much lesser degree. If a patient is seen relatively early in the course of the disease, Leydig cell function may be so little disturbed as not to be detectable clinically. The main distinguishing features common to all cases of prepuberal hyalinization of the tubules are: (a) azoospermia; ejaculation is usually normal but the seminal fluid is devoid of spermatozoa; (b) very small testes in a normally developed scrotum —testes are unusually firm and range in size from that of a pea to that of an average lima bean, although in rare instances, the testes are almost of

normal size; (c) there is a negative response to chorionic gonadotropin and urinary gonadotropins are elevated to the levels found in castrates; (d) testicular biopsy examination (10) reveals hyalinization of the tubules and aggregation of Leydig cells (Fig. 9) with an apparent increase in their total number (11).

Three clinical subtypes (9) are recognizable, depending apparently upon

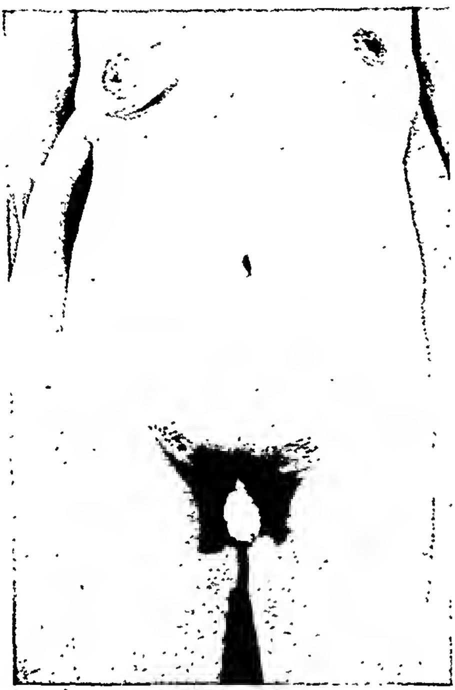

FIG. 10. Puberal seminiferous tubule failure—noneunuchoidal type.

the degree of involvement of the androgen-secreting cells of Leydig. These clinical types may be divided according to their appearance into: 1) the noneunuchoidal group[4] (Fig. 10) in which the patients invariably exhibit gross gynecomastia; 2) the moderately eunuchoidal group (Fig. 11) in which there is slight gynecomastia and a few eunuchoidal features; and 3) the eunuchoidal group (Fig. 12) in which the patients exhibit the features of classical eunuchoidism but lack gross signs, although they may have microscopic evidence of gynecomastia. It should be remembered that al-

[4] "Noneunuchoidal" has been substituted for "normal," as suggested by Bettinger and Robinson (12).

though the degrees of clinical hypogonadism and of Leydig cell failure are variable, seminiferous tubule failure is invariable; and members of all three groups have the findings associated with hyalinized tubules. It is presumed that the quality of the Leydig cells is poor in all cases since all of the patients we have observed developed symptoms of the male climacteric by the time they reached the age of 25 years.

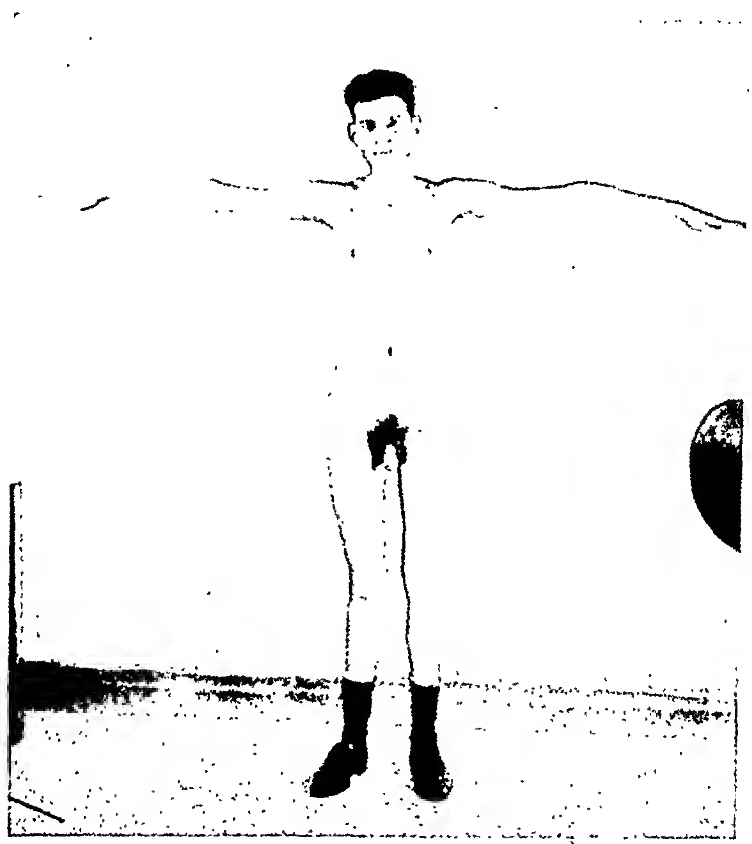

FIG. 11. Puberal seminiferous tubule failure—moderately eunuchoidal type. Note great similarity to normal male, but absence of facial and axillary hair, high-pitched voice, lima bean-sized testes, lack of recession of hair of forehead, and moderately eunuchoidal skeletal structure all aid in distinguishing from normal male.

We consider the defect in puberal seminiferous tubule failure to be of congenital origin. Reifenstein (13) has noted its occurrence in 9 members of the same family. The defect makes its appearance at the time of puberty as judged by clinical history, physical examination and histologic findings in the testis. All patients exhibit some degree of androgen secretion at puberty. In the noneunuchoidal group onset of the gynecomastia and atrophy of the testes may be traced to the time of puberty. The seminiferous tubules and Leydig cells both show evidence of puberal stimulation before the onset of failure.

Therapy. Neither the gyneconiastia nor the sterility (azoospermia) can be corrected hormonally. However, by instituting substitutional therapy with testosterone, as previously outlined, most gratifying increases in muscular strength and endurance, improvement in confidence, self esteem and reversal of neurotic tendencies result. Improvement in sexual function results as well (14).

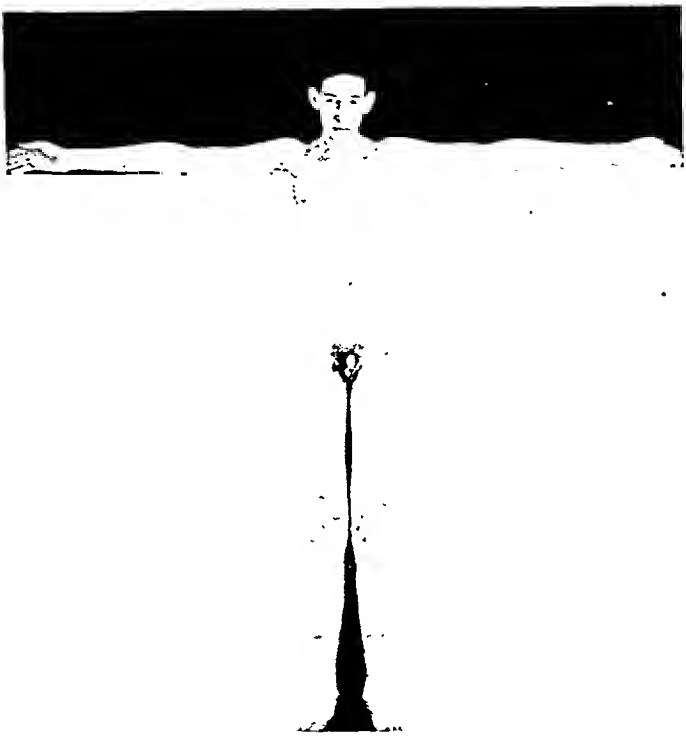

FIG. 12. Puberal seminiferous tubule failure—eunuchoidal type. Courtesy of Dr. D. R. Sparkman, Seattle.

4a) **Adult seminiferous tubule failure,** is usually discovered only in the course of an evaluation of instances of sterility. No clinical signs or symptoms are apparent. Seminal fluid examination reveals azoospermia or oligospermia. The testes are usually of normal size and consistency. If failure is complete, urinary gonadotropin levels are markedly elevated and testicular biopsies demonstrate either empty seminiferous tubules in which no elements of the germinal series are seen or hyalinization of the tubules (15). No effective treatment has been found.

12. Bettinger, H. F., and Robinson, B.: Klinefelter-Reifenstein-Albright syndrome, *M. J. Australia* **2**: 446–449 (Sept. 28) 1946.
13. Reifenstein, E. C. Jr.: Hereditary familial hypogonadism, *Proc. Am. Fed. Clin. Res.* **13**: 86, 1947.
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MALE PSEUDOHERMAPHRODITISM*

PROVED BY SURGICAL EXPLORATION AND MICROSCOPIC EXAMINATION

A CASE REPORT WITH SPECULATIONS CONCERNING PATHOGENESIS

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WHEN an unusual case presents itself and challenges the validity of current concepts for its explanation, one should be prepared for surprises. The following case found us totally unprepared for the answer.

CASE RECORD

J. W., a 19-year-old girl of Scandinavian-Scotch-Irish ancestry, was referred for study because she had never menstruated. She was the middle one of three sisters in a family in which the only suggestion of endocrine abnormality was the occurrence of unusually tall women (6 ft.) on the maternal side.

Born at term of an uneventful pregnancy, the patient had been a healthy, apparently normal girl who had had the usual quota of uncomplicated childhood diseases. The first sign of puberty appeared at 15 years when her breasts began to develop. Two years later she had attained a height of 69 inches and required a size 34 brassiere. The fact that she had not menstruated did not seem significant as her mother's menarche did not occur until her seventeenth year and the elder sister's in her sixteenth year. When at 18 years of age the menses had not appeared, the patient sought medical aid and consulted one of us (A. F. M.). Pelvic examination revealed a rudimentary vagina and the absence of uterus and cervix. Ethinyl estradiol, 0.1 mg. daily for two weeks each month, was prescribed. This therapy, continued for four months, resulted only in increased size of the breasts. Further investigation was interrupted when the patient developed a pleural effusion with fever, cough and night sweats. A diagnosis of tuberculous pleurisy necessitated six months' hospitalization at the San Fran-

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cisco Tuberculosis Hospital during which time hormone treatment was suspended. In February, 1946, she was discharged from the hospital as cured, and soon after was referred for further study of her endocrine condition.

When seen on April 30, 1946, the patient appeared to be a tall, athletically built, well nourished girl with relatively long arms and legs, a prominent lower jaw and rather large breasts (Fig. 1). Her voice was pleasantly feminine though of low pitch. The blood pressure was 120/60. Her measurements were as follows:

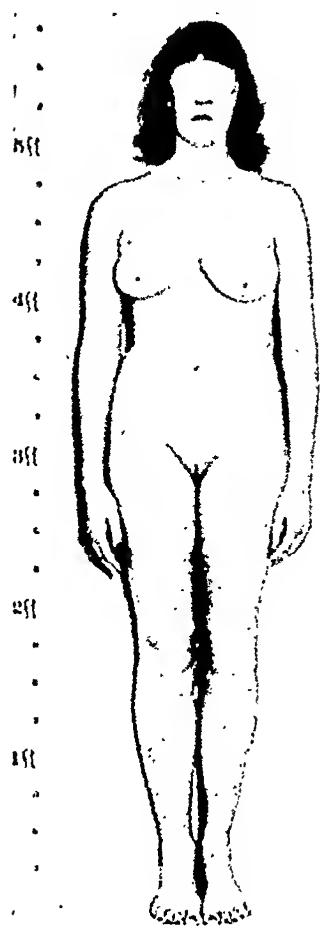

FIG. 1. J. W., aged 19. Note tall eunuchoid build, absence of pubic hair, large, well-developed breasts.

Weight	159 lbs.	(72.3 Kg.)
Height	70½ inches	(79 cm.)
Span	73½ inches	(86.7 cm.)
Vertex to pubis	33½ inches	(85.1 cm.)
Pubis to floor	37 inches	(94.1 cm.)
Ratio upper/lower	0.90	—

The skin was soft, smooth, and hairless. Pubic and axillary hair was absent but a slight trace of vulval hair was discernible. The labia majora and minora were normally developed but small. A perforate hymen spanned the vagina which ended blindly at a depth of 2.5 cm. The urethra occupied its usual position but the clitoris was represented by only a dimple. On palpation no pelvic organs could be felt. The other noteworthy positive physical findings were: a narrow mandible with crowded lower teeth; a high arched palate; a maldevelopment of the ear lobule on the left; slight asymmetry of the chest with dullness and absent breath sounds over the right side posteriorly; large full breasts with prominent areolae but small poorly developed nipples.

Laboratory data: Routine blood count, urinalysis, and blood serology were negative. Urinary gonadotropin assay (F.S.H.)[1] showed at least 96 m.u./24 hours but not as much as 192 m.u. (normal values: 13 to 50 m.u.). Vaginal smears stained with Shorr's trichrome stain showed fully cornified epithelial cells indicating estrogen effect.

X-ray findings: Roentgenograms of the skull demonstrated the extreme narrowing of the lower jaw but were otherwise normal. Those of the skeleton revealed a bone age of 19 to 20 years with no further potential for linear growth remaining. No osteoporosis or abnormal calcifications were noted.

Preoperative consideration: What were the diagnostic possibilities? Here was a tall 19-year-old girl with eunuchoid proportions, poorly developed external genitalia, absent uterus and cervix, moderately high urinary F.S.H. excretion but with large breasts. To explain the last, it seemed reasonable to suppose that some functioning ovarian tissue must be present despite the fact that the adnexa were nonpalpable. Since the patient was contemplating matrimony and wished a plastic operation performed on the vagina, it was decided first to determine her gonadal status either by peritoneoscopy or by pelvic laparotomy. The latter course was chosen because of previous unreliable results with peritoneoscopy in our hands.

Surgical exploration, December 16, 1946: Under sodium pentothal and cyclopropane anesthesia, the abdomen was opened through a midline incision. Exposure of the pelvis (Fig. 2) revealed no structures between the rectum and the bladder other than a fold of peritoneum which formed a shelf-like projection. Laterally and quite high in the pelvis were two shiny grayish-white, ovoid bodies, one on each side, measuring about 2.5×1.5 ×1.5 cm. Contiguous with the distal ends of these bodies were two fleshy, pyriform structures resembling the unfused horns of a uterus. The thickened fold of peritoneum stretching from these structures to behind the

[1] Method of Klinefelter, Albright and Griswold (1) (slightly modified).

bladder suggested round ligaments. Adjacent laterally to the rudimentary gonads were apparently normal tubes with fimbriated ends adherent to the upper poles. Folds of peritoneum corresponding to the uterosacral ligaments were well developed.

Although the gonadal structures had the innocent appearance of aplastic nonfunctioning ovaries, a biopsy seemed indicated. As the scalpel cut through the thick white capsule, it revealed unsuspected dark brown tissue (Fig. 3). Frozen sections showed the tissue to be composed entirely of

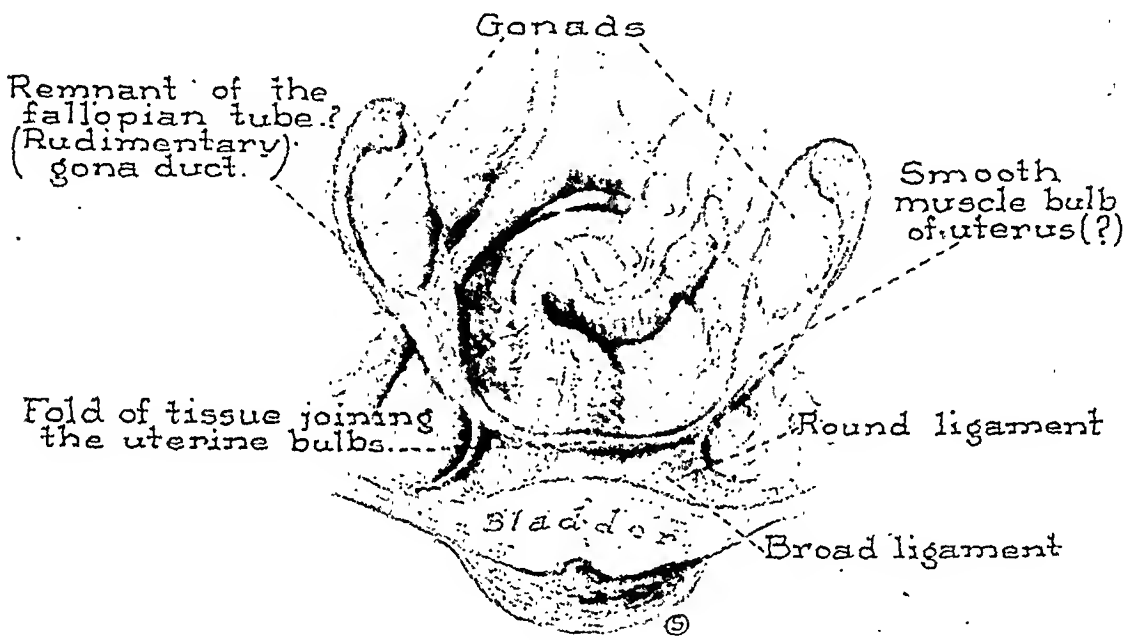

Fig. 2. Drawing of pelvic organs as seen at surgical exploration.

seminiferous tubules identical with the picture of Pick's "adenoma tubulare testiculare." A tentative diagnosis of arrhenoblastoma was made. The second "gonad" on section displayed the same gross appearance as the first. Both gonads with their respective tubes and the left rudimentary uterine horn were removed as well as the appendix. Numerous congenital bands were found around the appendix and the ileocecal valve. Exploration of the upper abdomen revealed a right adrenal which, through the peritoneum, seemed to be about the size of an almond. The left adrenal was somewhat smaller.

Subsequent course: 17-Ketosteroid excretion in the urine had not been determined prior to operation. A belated 24-hour specimen collected immediately following surgery assayed 7.2 mg./24 hours.[2] This is well within

[2] Method of Pincus (2).

the normal range. The postoperative course was uneventful. Six weeks later the patient reported that she felt well although she had lost about ten pounds in weight and was somewhat nervous. The only notable physical change other than a pallid appearance was that the breasts seemed smaller and flabbier. Vaginal smears showed atrophic cells and many leukocytes. Not until three and a half months postoperatively did the patient volunteer the information that she was having hot flashes and increased perspiration. Urinary gonadotropin assay at this time was positive

FIG. 3. Bisected right gonad. Note rudimentary fold suggesting gonaduct.

for 195 m.u./24 hours. Oral administration of ethinyl estradiol 0.05 mg. daily produced prompt relief of symptoms.

Psychologic aspects: Inquiry into the psychologic aspects of this case brought out the following information: The patient was of an affectionate nature and enjoyed manifestations of affection from the opposite sex. However, she had always felt herself to be different from other girls in a very indefinite sort of way. Whether this was due to her physical difference or to an actual psychologic difference is impossible to say. Since the operation she is more than ever anxious for marriage and seems cheerful, happy and full of vitality.

Gross pathology: The material received consisted of two smooth, bluish white masses. One was from the right and the other from the left posterolateral pelvic wall, with elongated rounded folds attached to each mass. There was a separate mass from the left posterior pelvic wall and an appendix. The right bluish white mass was $2.7 \times 2 \times 1.5$ cm. On section the surface was lobulated, soft, and brown in color with a scattering of firmer pale yellow discrete nodules from 0.4 to 1.4 cm. in diameter (Fig. 4). The

left mass was 3×1.5×1.2 cm. On section, the surface was similar to that of the right mass except that only one discrete yellow nodule 0.5 cm. in

FIG. 4. Cross section of right gonad. ×10. Note thick tunica and discrete adenoma.

FIG. 5. Cross section of left gonad. ×120. Note small calibered tubules and islands and strands of interstitial cells.

diameter was apparent. The cylindrical structures were 2.5 cm. long and 0.5 cm. in diameter; a narrow longitudinal area was dissected but otherwise the structures were covered by peritoneum. The separate piece of tissue measured 2×2×1 cm.; one surface was white and glistening whereas

the opposite surface was dissected. On section the surface had a faintly striated pattern.

Microscopic pathology: The two bluish white masses were similar in structure (Fig. 5). Each was covered by a collagenous fibrous tissue capsule

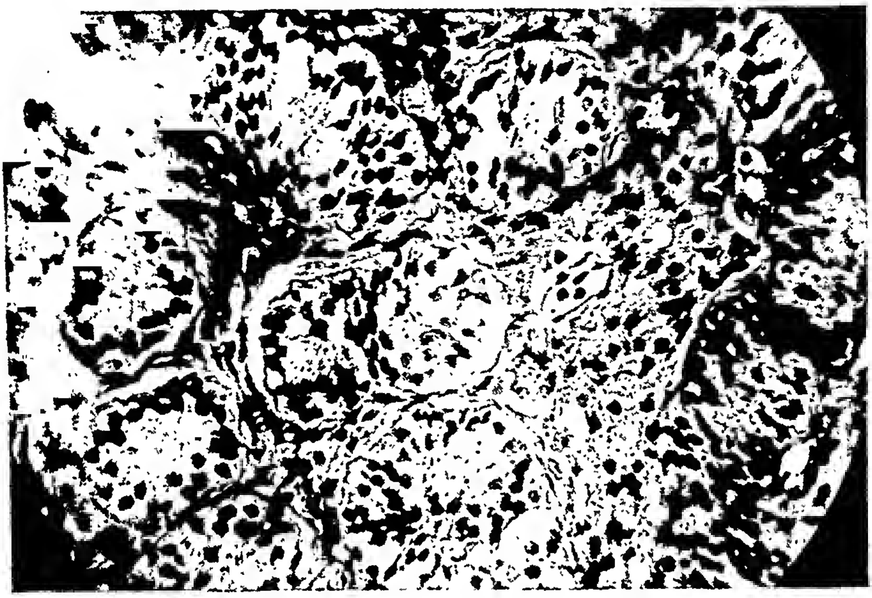

FIG. 6. Cross section of left gonad. Note immature tubules, absence of spermatogenesis and islands of interstitial cells.

from 2 to 3 mm. thick. Narrow fibrous septa containing irregularly distributed groups of cells morphologically resembling Leydig cells divided the soft brown tissue into irregular lobules. This tissue was made up of cords which varied considerably in structure but in general resembled immature seminiferous tubules. The narrower cords had a central mass of basophilic, granular material and a single layer of peripheral round nuclei with loosely spaced chromatin granules. The centers of the wider cords were paler staining and contained more deeply staining radial strands and small, faintly basophilic ovoid bodies. In these cords the cell membranes were often indefinite, the cytoplasm pale staining, and the nuclear chromatin more distinctly granular. The nuclei were all uniform in size and none of the cells displayed the characteristics of spermatogonia or Sertoli cells[3]

[3] A portion of the tissue fixed in formalin was later sent to Dr. Warren O. Nelson of the Medical School of Iowa State University for additional study. His report corroborated the fact that the overwhelming majority of the tubular cells were relatively undifferentiated. However, by special staining technic he was able to identify occasional Sertoli cells.

(Fig. 6). There were no rete tubules. Sections at various levels of the folds which suggested gonaducts showed only fat covered by a thin layer of mesothelium. No epithelial structures were evident. Sections of the cephalic end of one of the rounded folds, however (Fig. 7), showed an indentation lined by long narrow plicae covered by ciliated high columnar epithelium. At the open end, a wider plica on one border resembled a fimbria. The wall was composed of fibrous tissue with irregularly distributed smooth muscle

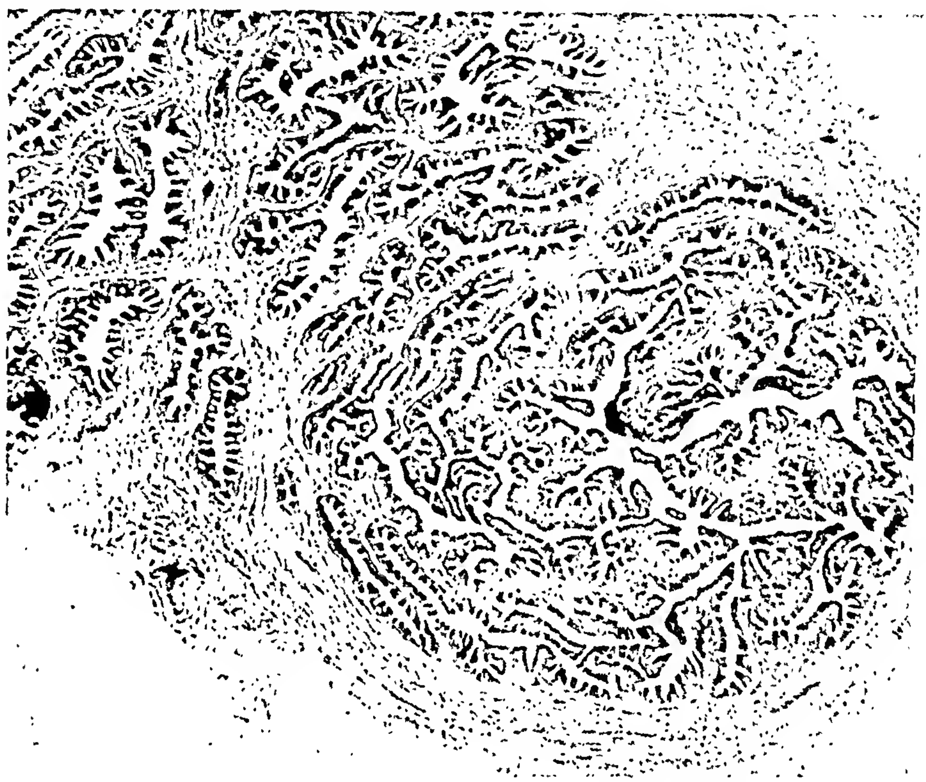

Fig. 7. Cross section of cranial portion of rudimentary tube. ×70.

bundles. The separate mass from a lower level was composed of interlacing bundles of smooth muscle fibers containing some nerve structures and sinusoids characteristic of myometrium (Fig. 8). No epithelial components were noted in any of the sections of this structure.

Pathologic diagnosis: 1) Fetal testes with adenomata, and hyperplasia of interstitial cells. 2) Wolffian agenesis. 3) Rudimentary Müllerian derivatives.

DISCUSSION

Although the preliminary diagnosis of adenoma tubulare testiculare of Pick (3) based on the microscopic picture was correct, the assumption that this represented a highly differentiated form of arrhenoblastoma of the

type described by Meyer (4) was in error. Careful search both grossly and microscopically failed to reveal any vestige of ovarian tissue. Obviously the diagnosis was neither arrhenoblastoma nor ovotestis. These gonads were immature, sterile, cryptorchid testes showing the typical adenomatous formation, small calibered tubules without spermatogenesis, and increased development of interstitial cells described over and over again in the literature (3, 5, 6, 7). This girl (?) then was an intersexed individual represent-

FIG. 8. Cross section from uterine anlage. ×70.

ing another instance of male pseudohermaphroditism with Müllerian derivatives and complete agenesis of the Wolffian system.

Incidence: Why did we fail to recognize the true situation at the time of operation? Pseudohermaphroditism is not such a rare condition. According to Young (7) the incidence is as high as 1 in 1000 individuals. The literature is full of descriptions of various types of intersexuality. Clinical recognition of these has depended for the most part on the presence of external genital anomalies. Surgical repair of hernias containing anomalous gonadal structures and laparotomies for intercurrent conditions have brought to light other instances but the condition has often remained unsuspected throughout life only to be revealed at necropsy. This is particularly true of the type of case described herein, exhibiting 1) completely

feminine habitus without any signs of masculinization, 2) amenorrhea accounted for by the absence of a normally developed uterus, and 3) testes in lieu of ovaries in the *intra-abdominal* position.

It is not the purpose of the authors to tabulate all the recorded cases of this kind. Suffice it to say that a review of hundreds of instances of pseudohermaphroditism in the available literature brought to light only 7 cases which fulfill the above criteria (5, 8, 9a, 9b, 9c, 10, 11). That the incidence is undoubtedly much greater than the above figure would indicate seems likely, as many such individuals probably go through life unsuspected, undetected and unreported.

Pathogenesis: When we come to a consideration of the pathogenesis of this condition, we enter into a field of pure speculation. Is it due to a genetic or chromosomal fault at conception or to some modifying environmental influence in early embryonic life? Although at the present time this question cannot be answered definitely for the human being, a brief review of some of our present concepts of the genetics and embryology of sex in correlation with the findings in this case may throw some light on this interesting problem.

From the genetic standpoint, the sex of a zygote is determined at fertilization by the chromosomal content of the fertilizing sperm. The mechanics is uncertain but it is generally accepted that the genes synthesize enzymes which control the differentiation of tissues. The balance between the autosomes and a single X chromosome results in a male embryo, two X chromosomes being necessary to produce a female. According to Goldschmidt's theory (12), this differentiation is quantitative rather than qualitative and any quantitative alteration in balance between X chromosomes and autosomes may give rise to various degrees of intersexuality. So much for the genetic factors. Progressing then from the conditions prevailing at conception, the next step is embryologic development. In the human being this is divided into three main stages: 1) the period culminating in the implantation of the blastocyst, or the first three weeks; 2) the embryonic period, from the beginning of the fourth week to the end of the eighth week, characterized by rapid growth and differentiation of tissues. It should be noted that during this period all the main systems and organs and the major features of the external body form are established; and 3) the fetal period from the ninth week to term characterized more by added increment of growth than by tissue differentiation (13).

Through the first seven weeks of gestation there is no discernible difference between the male and female embryo. Sexual differentiation commences at this time (17 mm.) and is usually complete by nine weeks (33 mm.). Development of the gonads and that of the gonaducts proceed independently of each other at this stage under genetic control (14). This is well illustrated by the perfect development of the Müllerian system in those

cases with absent or aplastic ovaries (15). Fusion of the Müllerian ducts occurs at eight weeks (25 mm.). This failed to occur in our patient. The gonad of the male embryo at 20 mm. shows beginning interstitial cell formation but the tubules are composed entirely of small undifferentiated testicular cells. Spermatogonia can be differentiated at 31 mm. (16). In our patient the interstitial cells were well developed but no spermatogonia were found. At eight weeks (25 mm.) the genital tubercle becomes bent caudally and can be recognized as the clitoris. In our case the clitoris failed to develop. Concerning the origin of the vagina there is some difference of opinion. Koff (17) claimed that approximately the lower one-fifth of the vagina is derived from the endoderm of the urogenital sinus. This would explain the perfect formation of the hymen and lower portion of the vagina in our case even though the remainder of the structure failed to develop.

Recapitulation of the evidence suggests, but does not prove, that a genetic fault had been responsible for a confused differentiation of the genital system and that this differentiation, but not growth, had ceased at about the eighth week of gestation. All but two of the anatomic discrepancies or anomalies in this case have been accounted for. The two remaining to be explained are the absence of axillary and pubic hair and the presence of large well-developed breasts.

The significance of the increased development of interstitial cells in cryptorchid testes and in the testes of pseudohermaphrodites has not been satisfactorily explained. If the Leydig cells are the source of the androgenic hormone, as is claimed, one would expect increased masculinization under these circumstances. Such definitely was not the case in this patient. The observations of Cole et al. (18) are of interest in this respect. They reported that in the fetal horse, the testes, which are composed predominantly of masses of interstitial cells, showed a very high concentration of estrogens. Are these cells then the source of the estrogens responsible for the breast development and the cornified vaginal smears? The relatively small amount of estrogen therapy one and one-half years prior to surgery played very little part, as the breasts had been well developed before treatment was instituted. Were the adrenals responsible? Perhaps, but the evidence again indicates that the testes, immature as they were in respect to spermatogenesis, were capable of hormonal activity. Following castration not only did the urinary gonadotropin titer rise and flushes and sweats appear but the breasts definitely became smaller and flabbier; and the vaginal smear, previously normal, showed atrophic cells. The question next arises as to the reason for the moderately increased urinary F.S.H. value in this patient prior to castration. For this we have no explanation to offer unless it be the absence or paucity of Sertoli cells (19). The lack of sexual hair remains to be explained. None has developed under estrogenic ther-

apy. Androgenic therapy has not been tried nor has a skin biopsy been done to ascertain the presence of hair follicles.

Was the castration in this case justifiable? Under the working diagnosis at the time of surgery there was no alternative. In the light of our later conviction it is still justifiable because 1) there was removed prophylactically a statistically important focus for the development of new growths. The occurrence of neoplasms in cryptorchid testes and the gonads of known pseudohermaphroditic individuals is at least twenty times more common than in the population at large (4, 7); and 2) the presence of these abnormal immature gonads contributed nothing which substitution therapy would not adequately provide.

Finally we are faced with the philosophical question: into what category of sex shall we place this individual? Psyche and external habitus were predominately female. Histologically the gonads were male—all male. Yet these testes, from the standpoint of spermatogenesis, had never advanced beyond the fetal stage and hormonally would seem to have exerted more estrogenic than androgenic effects. Upon what, then, does the ultimate basis for the determination of sex depend?

SUMMARY

A case is presented of a 19-year-old girl, tall, with eunuchoid proportions, poorly developed external genitalia, rudimentary vagina, absent clitoris, absent uterus and cervix, absent axillary and pubic hair, moderately high urinary gonadotropin titer and large breasts. Surgical exploration revealed two rudimentary gonads with "attached tubes" and two unfused uterine anlagen. Biopsy of the gonads showed the typical picture of Pick's "adenoma tubulare testiculare." The gonads with their attached tubes and one uterine bulb were removed. Microscopically, the gonads proved to be fetal testes with adenomata, immature seminiferous tubules and a hyperplasia of interstitial cells.

An attempt is made to place the exact time in embryonic life at which all the anatomic deviations occurred. This would appear to be during the eighth week of gestation and to be most likely on a genetic basis.

Evidence is offered to suggest that the immature testes had been hormonally active and that this activity was of an estrogenic rather than androgenic nature.

ACKNOWLEDGMENTS

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THE RELATION BETWEEN INFANT BIRTH-WEIGHT AND SUBSEQUENT DEVELOPMENT OF MATERNAL DIABETES MELLITUS

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THAT diabetic women frequently give birth to unusually large infants is well known. Only recently, however, has it been emphasized that infants of excessive weight may be born to mothers *before* clinically apparent maternal diabetes mellitus develops (1). Thus, the average weight of infants born to prediabetic mothers considerably exceeds the normal (2). The combined fetal and neonatal mortality rate is high in the prediabetic group as well as in the diabetic (3), and similar fetal, visceral changes are present in both groups (4). This study was undertaken to ascertain how the birthweight of an unusually large baby can be correlated with the likelihood of the subsequent development of maternal diabetes, and to determine the average time elapsing between the birth of a large baby and the development of clinical diabetes.

METHODS

One hundred parous, diabetic women with an average age of 55.3 years, who remembered the birthweights of their children, constitute the diabetic group (Group 1). These individuals were outpatients of the Washington University Diabetic Clinic or were hospitalized on the medical service of the Barnes Hospital. An equal number of parous women without glycosuria or symptoms of diabetes, from the medical and surgical public wards, served as controls (Group 2). The controls were also largely beyond the childbearing age; their average age was 51.2 years. Each group was asked for the following information:

1. Present age of patient.
2. Age when diabetes developed (diabetics only).
3. Number of pregnancies.
4. Birthweights of each child.
5. Family history of diabetes (patient's and husband's family).
6. Birthweights of the patient and near relatives.
7. Age of the patient at the time of delivery of her first "big baby."

Miscarriages, deaths in utero and premature deliveries were excluded

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from all tabulations and computations involving Groups 1 and 2. The age at the time of development of diabetes was chosen as that year in which the condition was definitely diagnosed by a physician. Any infant weighing 10 pounds or more at birth was termed "big"; any infant weighing less than 10 pounds was termed "normal" (5). None of the babies in this study was born to a mother clinically diabetic at the time of childbirth. In order to obtain data on a larger group of births, the birthweights of 1000 consecutive babies whose births were recorded as occurring at full term in the St. Louis Maternity Hospital in the year 1927 were used as additional controls (Group 3). The year 1927, although subsequent to the average date of birth of the infants in Groups 1 and 2, was the earliest year for which data were readily available. In compiling the data for Group 3, no attempt was made to exclude from the series births to diabetic women.

RESULTS

Distribution of big infants: Three hundred and sixty children were born to the 100 prediabetic mothers, and 315 were born to the control women of

TABLE 1. BIG INFANTS BORN TO 100 PREDIABETICS AND TO 100 CONTROLS (GROUPS 1 AND 2)

Infant Weight, lbs.	Total Number of Big Infants	Status of Mother					
		Prediabetic		Control, with Positive Family History of Diabetes		Control, with Negative Family History of Diabetes	
		Number of Big Infants	Percentage of Big Infants	Number of Big Infants	Percentage of Big Infants	Number of Big Infants	Percentage of Big Infants
10 or more	144	111	77.1	10	6.9	23	16.0
11 or more	78	68	87.2	4	5.1	6	7.7
12 or more	52	47	90.3	2	3.9	3	5.8
13 or more	24	23	95.8	1	4.2	0	0.0
14 or more	12	12	100.0	0	0.0	0	0.0
15 or more	3	3	100.0	0	0.0	0	0.0

Group 2. A striking correlation between the birth of big babies and the prediabetic state is shown in Table 1. Of the 144 infants weighing 10 pounds or more, 77.1 per cent were born to women destined to develop diabetes. An additional 6.9 per cent were born to Group 2 (control) women with a positive family history of diabetes, whereas only 16.0 per cent were born to Group 2 mothers with a negative family history. Compared to the control series, the preponderance of large infants coming from prediabetic

women increased as the birthweight rose. For example (see Table 1), 87.2 per cent of babies weighing 11 pounds or more were born to prediabetics, and only 7.7 per cent were born to normal women from a nondiabetic family. At the level of a birthweight of 13 pounds or greater, 23 of 24 infants, or 95.8 per cent, came from prediabetic mothers.

Frequency of big infants: Fifty-eight per cent of the diabetic group gave birth to at least one big baby during their prediabetic years. Many prediabetics gave birth to more than one big infant; thus, 111 big babies were born to 58 women. Twenty-seven per cent of the control group gave birth to at least one big baby, but 8 of the 27 were mothers with diabetes in their family. When all those families with diabetes are excluded, it was found that only 24.4 per cent of the remaining group have had at least one large baby. Twenty-six of the 100 diabetic women gave birth to *two* or more big infants. In contrast, only 5 of the Group 2 control women had two or more big infants, but diabetes subsequently developed in the sons of 2 of these 5 women. Thus, only 3 of the control group with a negative family history of diabetes gave birth to two large infants.

Birthweight distribution: The extent to which the prediabetic state influences the birthweight of the infant is also shown in Figure 1. The distribution curve of infant birthweights from the 100 mothers of the control group (Group 2) has its peak between 7 and 8 pounds, and rapidly falls off so that at a weight of 10 pounds only about 2 per cent of the group are included. The distribution curve for the children of the 100 prediabetic mothers (Group 1) is displaced toward the higher weights. Although the peak in this group also occurs between 7 and 8 pounds, the percentage of babies in the higher weight groups is considerably greater than in the controls. Additional data on birthweight are charted in Figure 1, as obtained from a study of 1000 unselected consecutive births occurring in the St. Louis Maternity Hospital in 1927 (Group 3). The birthweight distribution curve of the hospital births corresponds closely to the curve pertaining to the control group of 100 mothers (Group 2).

The average birthweight of children born to our prediabetic mothers was 8.9 pounds or 4.0 kilograms, whereas the average birthweight of the children of the control mothers (Group 2) was 8.0 pounds or 3.5 kilograms. Statistical analysis of the birthweight data shows that the standard error of the difference between the means of Groups 1 and 2 is 0.144 pounds. Calculated on the basis of probability, a difference of 0.9 pounds between means is statistically significant. Our data are, therefore, in agreement with those of Miller (2) in demonstrating that the average birthweight of children born to prediabetic mothers is greater than that of children born to normal mothers.

Latent period: In the 58 of the 100 prediabetic patients who gave birth

to big babies, the average age at the time of birth of the first big baby was 22.9 years. Diabetes was diagnosed at an average age of 47.1 years, and the "latent period" between the birth of the big baby and the development of clinical diabetes averaged 24.2 years. In this series the shortest latent

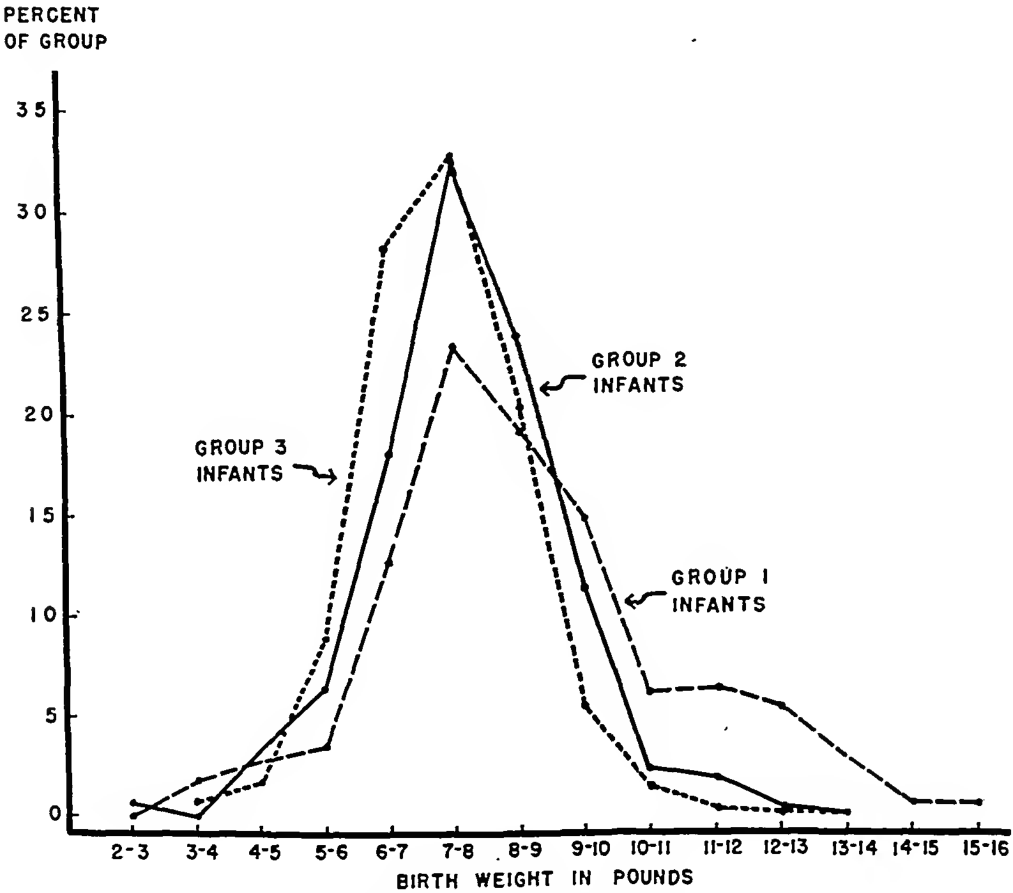

Fig. 1. Birthweight distribution curve of infants born to prediabetic and to control mothers. Dashed line: 360 births to 100 prediabetic mothers (Group 1). Solid line: 315 births to 100 control mothers (Group 2). Dotted line: 1000 consecutive full-term births, St. Louis Maternity Hospital, 1927 (Group 3).

period was 1 year and the longest was 46 years. Only 5 of the 58 patients had a latent period less than 10 years.

Diabetes mellitus developing in big babies: Four of the 144 big babies here reported are known to have developed diabetes at an average age of 18.8 years. In addition, 4 of the 100 prediabetic mothers were themselves known to have been big babies. Thus, there are 8 cases in which it is def-

initely known that big babies have developed diabetes. Three of these 8 had a negative family history of diabetes but a positive family history of big babies; these 3 developed diabetes at an average age of 27.0 years.

Prediction of maternal diabetes on basis of birthweight of offspring: The data thus far presented clearly demonstrate a relationship between unusually large babies and the prediabetic state. It is important to know, there-

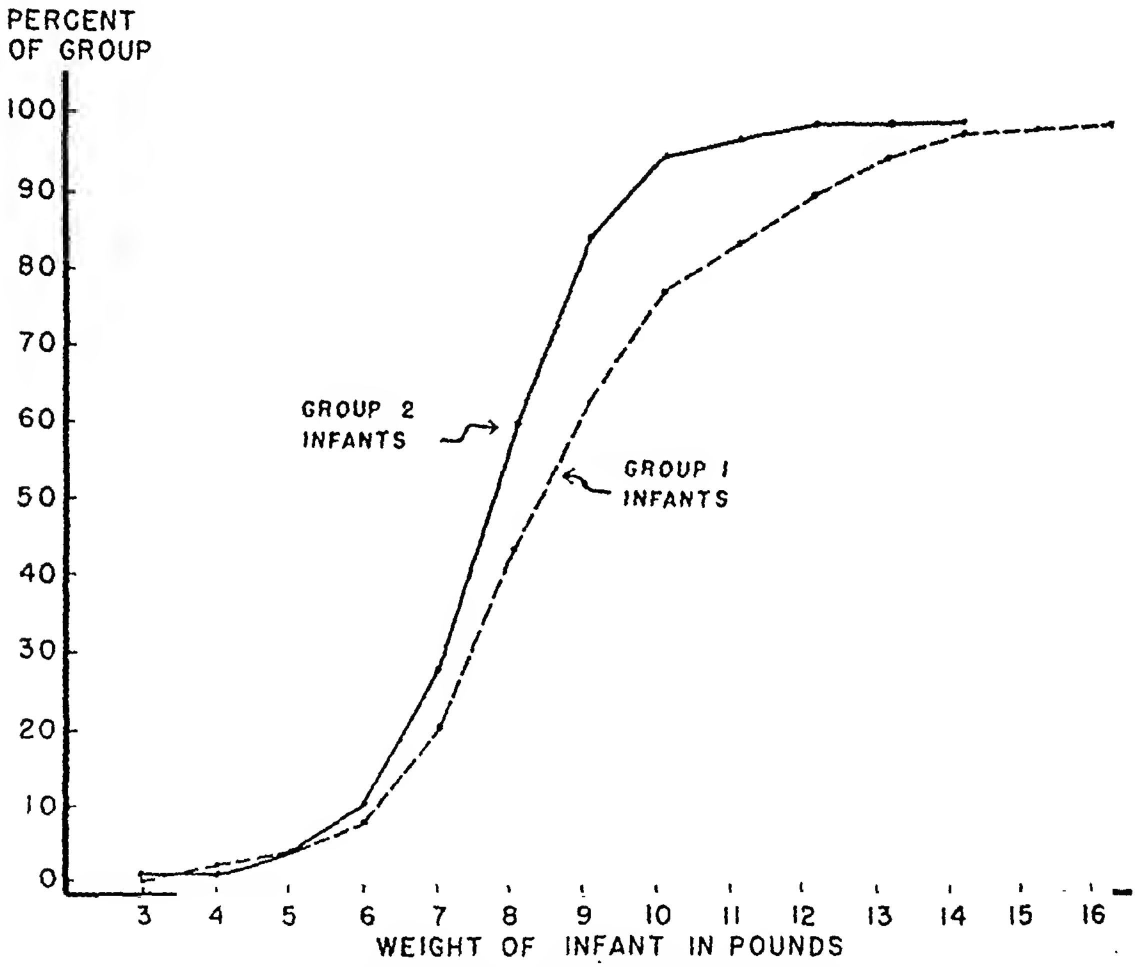

FIG. 2. Cumulative frequencies of infants born to prediabetic and control mothers correlated with birthweight. Dashed line: 360 children of 100 prediabetic mothers (Group 1). Solid line: 315 children of 100 control mothers (Group 2).

fore, whether one is justified in making a prediction of subsequent diabetes in a mother who bears a big baby. If the birthweight data from our diabetic women (Group 1) and our group of controls (Group 2) are used to construct a cumulative frequency distribution curve, or ogive (Fig. 2), the proportion of children above or below any given weight is readily obtained. These curves were constructed by calculating and plotting the percentage of infants weighing 3 pounds or less, the percentage weighing 4 pounds or less, and so on for each weight plotted. For example, from Figure 2 one learns

that 22.0 per cent of the children born to the prediabetic women weighed over 10 pounds, whereas only 4.5 per cent of children born to the controls were of this weight. The average age at which our prediabetic females gave birth to their first large baby was 23 years. A survey of large portions of the population indicates that at this age the chances of a woman eventually becoming diabetic are 42.3 per 1000 (6). If 100,000 women aged 23 years each gave birth to a child, 4230 children would be born to women destined to develop diabetes. According to our findings, 22.0 per cent of these children, or 933, would weigh over 10 pounds. Of the remaining 95,770 children whose mothers would not eventually develop diabetes, only 4.5 per cent, or 4300, would weigh over 10 pounds. If we were to have made the prediction of diabetes in the mother because she gave birth to a baby weighing over 10 pounds, we would have been correct 933 times and wrong 4300 times. Our prediction accuracy would, therefore, have been 17.8 per cent. A similar analysis may be made for higher birthweight groups. Assuming we predicted diabetes in the mother solely on the basis of the birthweight of her child, the prediction accuracy for a given birthweight is as indicated in the final column of Table 2. It is apparent that the prediction accuracy increases steadily as the birthweight increases. Since the chances of any woman aged 23 becoming diabetic are 42.3 per 1000, the prediction accuracy at this age is only 4.23 per cent (6), if one disregards the pregnancy record. However, when the woman's pregnancy record is considered, it can be seen from Table 2 that the prediction accuracy may be increased fourfold to fifteenfold over the usual accuracy, depending on the weight of the abnormally large infant. The calculations made for women aged 23 serve only as an example; the figures are roughly the same for women throughout the childbearing period, since the chances of a female becoming diabetic do not change appreciably until the age of 45 years (6).[1]

The figures in Table 2 should be regarded as only roughly approximate; they are based upon such assumptions as that prediabetic women have as many children as nondiabetic women, that diabetes will not develop subsequently in our control Group 2, and that the chance of developing diabetes is the same for parous women as for nulliparous women. It is uncertain whether our prediction accuracy figures based on women giving birth

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[1] "Prediction accuracy" calculations have also been made on the basis of the incidence of infants observed to be of a specific weight, rather than on the basis of the observed cumulative incidence of infants weighing over a specified weight as described above and presented in Table 2. The "prediction accuracy" figures for mothers bearing a single big infant, correlated with birthweight, when calculated in this manner are as follows: 10 to 11 pounds, 10.7 per cent; 11 to 12 pounds, 13.0 per cent; 12 to 13 pounds, 45.2 per cent; over 13 pounds, 100 per cent.

to babies in about the year 1920 will be equally applicable to mothers giving birth to babies in 1947. It is recognized that birthweight information obtained by interview is not as reliable as that obtained from hospital records. We obtained information from our diabetic women (Group 1) and our controls (Group 2) by identical methods in an attempt to control

TABLE 2. CALCULATED PROGNOSTIC SIGNIFICANCE OF A LARGE INFANT. PERCENTAGE OF MOTHERS AGED 23 YEARS AND BEARING BIG BABIES WHO MAY BE EXPECTED TO DEVELOP DIABETES MELLITUS SUBSEQUENTLY, CORRELATED WITH SIZE OF INFANT

Infant Birthweight in Excess of	Observed Incidence Births of Specified Weight to		Calculated Incidence of Births of Specified Weight to			"Prediction Accuracy"†
	Prediabetics	Controls	Prediabetic Mothers	Normal Mothers	Total Mothers	
Lbs.	Per Cent	Per Cent	per 100,000 births*	per 100,000 births*	per 100,000 births*	Per Cent
10	22.0	4.5	933	4300	5233	17.8
11	16.9	2.3	715	2200	2915	24.5
12	9.5	0.4	401	383	784	51.2
13	3.9	0.1	165	96	261	63.2

* 100,000 births to mothers aged 23, unselected as to normal or prediabetic maternal state; the term "normal" is employed to describe those mothers who will never develop diabetes mellitus.

† Calculated incidence of prediabetic state in mothers bearing baby of specified weight:

$$\left(\frac{\text{calculated prediabetic births}}{\text{calculated total births}} \times 100\right).$$

this factor. It should be added that the prediction accuracy refers only to the possibility of developing diabetes and does not relate to the prediction of *not* developing diabetes.

DISCUSSION

Both maternal and fetal factors must be considered in attempting to establish why fetal giants occur so commonly in the prediabetic state. It is well known that babies tend to be heavier with longer gestational age. Prolonged gestation has been shown (7) not to be common in the diabetic mother, and there is no reason to suspect it in the prediabetic. Maternal

obesity plays a role in the causation of large infants, according to Eastman (8); others assert that neither maternal obesity (2) nor maternal gain in weight during pregnancy (9) are causally related to fetal gigantism. Hyperglycemia in the mother and fetus has been excluded as a cause in the opinion of Miller (2) because, in a series of his prediabetic mothers bearing large babies, no evidence of diabetes presented itself on the basis of urine and blood sugar determinations at the time of delivery. Eastman (10) has often observed diminished glucose tolerance during the postpartum period in "normal" mothers bearing heavy infants. He does not believe, however, that maternal hyperglycemia is the sole cause of these large babies, or even the most important cause. Evidence discounting hyperglycemia as a factor is the finding that experimental diabetes in animals does not lead to fetal gigantism; Miller (11) has recently shown that the birthweights of rats, rabbits, and dogs are normal despite the presence of maternal diabetes induced by alloxan.

Consideration should be given to excessive production of maternal anterior pituitary growth hormone as a possible cause of fetal overgrowth, since hypertrophy of the anterior pituitary and histologic changes in several of its cell types are known to occur in normal pregnancy (5). On the other hand, there is evidence that the pituitary of the fetus itself may play a role in fetal overgrowth. Miller and his co-workers (4, 12, 13) have demonstrated, in babies born to prediabetics, increased numbers of eosinophil cells in the pituitary gland, splanchnomegaly, hyperplasia of the adrenals and islands of Langerhans, and hyperplasia of the female genital organs, all of which findings suggest anterior pituitary overactivity. Still another explanation for fetal gigantism, which allows for both maternal and fetal factors, has occurred to us. The fetus may have an inherited defect, possibly transmitted by *either* parent, which makes it unusually susceptible to normal stimulation by the maternal pituitary. Thus, although it may be true that maternal endocrine functions are responsible for the anatomic changes in the giant fetus, it does not necessarily follow that these maternal functions are abnormal, or that the assumed defect in the fetus is located in its pituitary.

SUMMARY

1. We have analyzed the birthweight data on infants born to 100 women destined later to develop diabetes mellitus and on infants born to two control groups.

2. Our findings, many of which corroborate the reports of others, lead us to conclude that:

a. The birth of an infant weighing over 10 pounds may herald the development of diabetes in the mother.

b. The average birthweight of infants born to prediabetic mothers is greater than that of infants born to normal mothers.
c. Women developing diabetes after the childbearing period are recruited to a large extent from those mothers who give birth to babies weighing over 10 pounds.
d. Abnormally large babies are born to prediabetic women more frequently than to normal controls.
e. The period between the birth of the first abnormally large infant and the development of clinical diabetes in the mother averaged about 24 years in our series, with a range of from 1 to 46 years.

3. On the basis of our data, a table has been constructed permitting rough estimation of the accuracy with which one may predict the subsequent development of diabetes in a woman giving birth to a baby having a specified weight in excess of 10 pounds. It shows that as the birthweight of the baby rises, the prediction accuracy increases progressively and is greater than 60 per cent when the baby weighs more than 13 pounds.

ACKNOWLEDGMENT

The authors are indebted to Dr. Willard M. Allen of the Department of Obstetrics and Gynecology, to Dr. Florence Heys of the Department of Pediatrics and to Mrs. Samuel Weissman of the School of Social Work of Washington University, and to Dr. N. J. Eastman of the Department of Obstetrics, Johns Hopkins University, for helpful suggestions in planning and reporting this work.

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EXTREME LEYDIG CELL HYPERPLASIA ASSOCIATED WITH TWO OTHER ENDOCRINE CHANGES*

A CASE REPORT

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INTRODUCTION

STUDIES of the Leydig cells of the testes have, to date, resulted in extreme confusion. Although the histology has been well described there are numerous and conflicting reports about their embryology, physiology and pathology. It would, therefore, be well to collect cases of the pathologic states of these testicular interstitial cells and to attempt to correlate them with some of the clinical endocrine changes.

CASE REPORT

The patient, a 54-year-old white farmer, developed hallucinations in 1929 and was first admitted to a neuropsychiatric hospital on June thirteenth of that year. His case was diagnosed as dementia *praecox*, hebephrenic type. He was single, and nothing unusual was noted about his sex habits. His use of alcohol was moderate, and he used snuff regularly. Venereal disease was denied. As a child he had mumps and pertussis. It is not known if there was orchitis with the mumps. He was said to have had small testes since childhood.

Physical Examination: At the time of admission his temperature was 98° F.; pulse, 90 per min.; blood pressure 100/62; respirations, 20 per min.; height, 70¼ inches, and weight, 163 pounds. He had black hair, blue eyes and a clear skin. He was well developed, and his musculature was good. Eye, ear, nose and throat examination showed only a slight hypertrophy of the left inferior turbinate. The thyroid gland was not enlarged. The heart, lungs and abdomen were normal. The testes were small and firm, no larger than those of a 10-year-old boy. The penis was normal in configuration and size, and the prepuce retracted well. No abnormalities of the extremities were noted. Both internal and external hemorrhoids were present.

Accessory Clinical Data: The complement fixation test for syphilis was

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negative. Urinalysis showed no sugar or albumen, in an acid urine of S.G. 1.027. The blood count was within normal limits.

In 1934 the patient was transferred to this hospital, and shortly thereafter a diagnosis was made of pulmonary tuberculosis, chronic, bilateral, moderately active, with cavitation in the left infraclavicular region. At that time results of the physical examination were essentially the same as five years previously, except for the chest findings. An examiner again

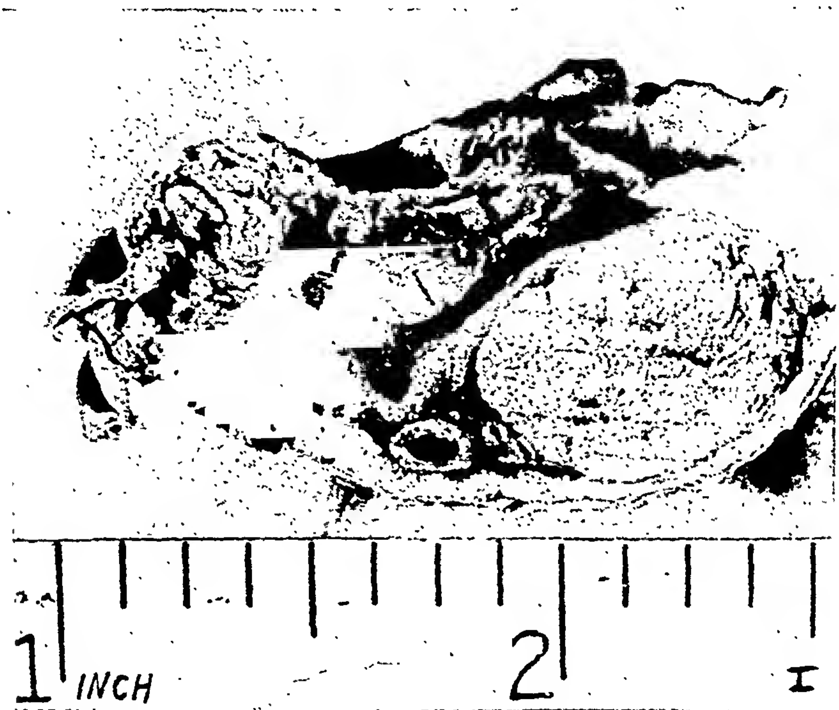

FIG. 1. Right adrenal, showing large cortical adenoma ($\times 2\frac{3}{4}$).

noted that the testes were small and undeveloped. The patient had well developed muscles, an average type of masculine voice and a male distribution of hair. It was noted that there were no evidences of endocrine disturbances.

The course of the tuberculous process was progressive. On March 3, 1947, the patient developed a tension pneumothorax and expired rapidly.

Autopsy Findings: (Only the pertinent findings are included.)

Gross Findings: The body was that of a well built, well nourished adult white male. The musculature was well developed. There was a normal male distribution of hair and no evidence of gynecomastia. In the pleural cavities there were dense, fibrous adhesions at both apices. Most of the

left lung was atelectatic and there was a large cavity and numerous smaller cavities in the apex. The entire right lung was atelectatic. There was an acute bronchitis, and mucopurulent material occluded the smaller bronchi in both lungs. The left adrenal gland was normal grossly. The right adrenal contained a well circumscribed cortical adenoma, 1.7 cm. in diameter (Fig. 1). It was bright, golden yellow and showed no areas of hemorrhage or necrosis. The adenoma was well demarcated from the adjacent cortex. The penis was well developed. The scrotum contained two small testes approximately equal in size. Only the right testis was removed and this measured

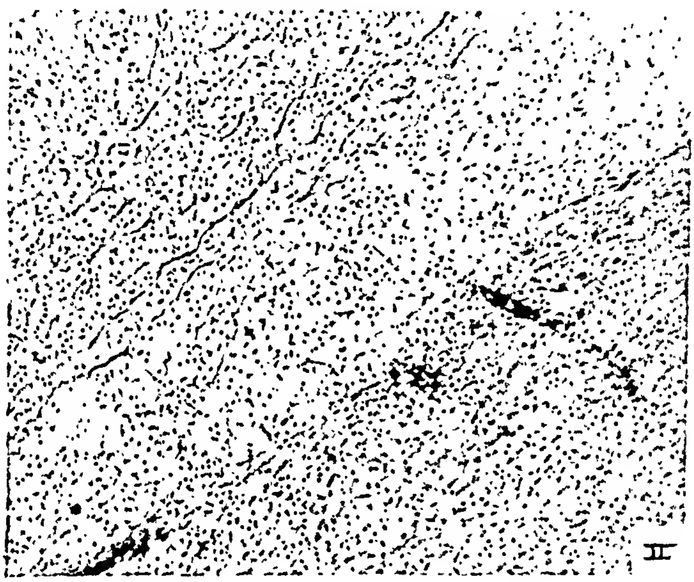

FIG. 2. Junction of adenoma with adrenal cortex. Hematoxylin and eosin (×60).

15×12×10 mm. It was firm and cut with moderate resistance. The tubules could not be strung out. The prostate was small and firm. The brain and the pituitary gland grossly showed no lesions.

Microscopic Findings: Sections of the lungs showed large areas of caseous necrosis with tubercle formation. There was much scarring, atelectasis, and some small patchy areas of emphysema. The larger bronchi contained purulent material in the lumina, and many mononuclear cells in the walls obscured the structure. There was destruction of the mucosa of a bronchus lying adjacent to an area of caseous tuberculosis.

Section of the left adrenal showed only some delipoidization of the cortex. The right adrenal contained a large oval mass separated from the rest of the cortex by thin strands of fibrous tissue (Fig. 2). The mass was composed of cords of large polyhedral cells with a clear cytoplasm and small dark nuclei. The mass was very vascular and was a well organized tissue.

The cortical cells of this adenoma and the remainder of the adrenal cortex showed delipoidization.

Section of the right testis showed no evidence of spermatogenesis. Numerous sections showed only occasional tubules (Fig. 3). The basement membranes of some of these tubules were hyalinized and thickened. The epithelium lining the tubules was tall and irregular in shape with clear cytoplasm and small pyknotic nuclei. The entire bulk of the testis was composed

FIG. 3. Section of right testis showing marked interstitial cell hyperplasia and absence of tubules. Hematoxylin and eosin (×60).

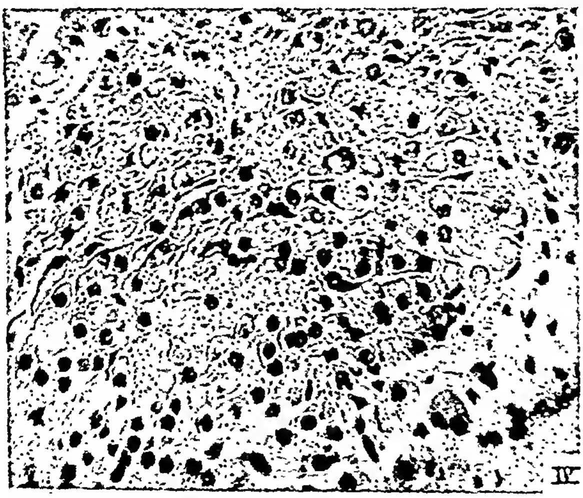

FIG. 4. Interstitial cells of the right testis. Hematoxylin and eosin (×244).

of numerous cell masses separated by small amounts of loose fibrous tissue (Fig. 4). There was no definite relationship of these cell masses to the blood vessels. The cells comprising these clumps were large, round and irregularly polyhedral. The nuclei were large, spherical, wrinkled and contained coarse chromatin granules and one or two large nucleoli. Some cells were binucleated. The cytoplasm of these cells was eosinophilic and in many instances contained a large amount of granular brown pigment. Crystalloids and refractile lipoid granules were not seen. The tunica albuginea and epididymis showed no interstitial cells and no changes. Sections of the prostate gland showed no changes.

TABLE 1. DIFFERENTIAL CELL COUNT OF PITUITARY GLAND

Cells	Cell Count		Per Cent of Total
A. Total cell count			
Normal alpha cells	320		
Pyknotic alpha cells	136		
Total alpha cells		456	34.7
Normal beta cells	124		
Pyknotic beta cells	47		
Degranulated beta cells	37		
Total beta cells		208	15.8
Total chromophobe cells		651	49.5
Total cells—all types		1315	100.0
B. Normal cell count			
Normal alpha cells	320		29.2
Normal beta cells	124		11.3
Normal chromophobe cells	651		59.5
Total normal cells		1095	100.0

A differential cell count was done on the pituitary gland.[1] The findings are given in Table 1 and they are analyzed in two ways: (A) the "total cell count," which may show the preterminal distribution; and (B) the "normal cell count" (undegenerated cells) which may show the terminal condition. It is not possible to say how long the preterminal condition existed. If one assumes that the pyknosis is not a postmortem change (the patient was embalmed immediately after death and the autopsy was performed

[1] By Dr. H. N. Marvin, Department of Anatomy, University of Arkansas.

seven hours later), then the normal cells plus pyknotic cells in each group gives the preterminal distribution. This showed a mild basophilic hyperplasia which is considered compatible with deficient production of male sex hormones. On the other hand, 24 per cent of the beta cells and 28 per cent of the alpha cells are pyknotic. This shows that the alpha and beta cells were undergoing degeneration at the same rate at the time of the autopsy. This could reasonably be interpreted as a degeneration resulting from terminal collapse. The results of the pituitary differential cell count are suggestive of hypogonadism.

Anatomic Diagnosis: Bilateral caseous tuberculosis with cavitation on the left; fibrous pleural adhesions; old and recent needle punctures in the thoracic wall, bilaterally; bilateral pneumothorax and bilateral compression atelectasis. Acute and chronic bronchitis with ulceration and mucopurulent material occluding the bronchi. Acute congestion and edema of the liver with beginning necrosis. Acute congestion of the spleen, fibrous peritoneal adhesions, Cortical adenoma of right adrenal. Atrophy of the testes with hyperplasia of the interstitial cells. Mild basophilic hyperplasia of the pituitary gland. Small scar in medulla and retention cysts of the left kidney.

DISCUSSION

The case presented shows more than a simple hyperplasia of the interstitial cells. The hyperplasia here is so great that the question of neoplasia may be legitimately raised. There is destruction and replacement of the tubules by the interstitial cells. This patient does, however, show those clinical findings ordinarily associated with hyperplasia, namely, small testes, age past 45 years, and the presence of a chronic debilitating disease.

There are associated endocrine factors in this case. There is an increase in the basophilic cells of the pituitary. The cortical adenoma of the adrenal is well formed and morphologically appears capable of being a functional tissue. The exact role of the adrenal in relation to the testis and pituitary is purely speculative.

An interesting feature of hyperplasia of the interstitial cells of the testis is the lack of associated virilism. Most patients with this change appear to be either normal or slightly eunuchoid (1) (2). The small testes are due to atrophy of the seminiferous tubules which comprise the greatest mass of testicular tissue.

The effect of interstitial cell tumors is not consistent. A case of gynecomastia associated with an interstitial cell tumor is reported by Hunt and Budd (3). On the other hand, three cases of precocious puberty occurring in cases of interstitial cell tumors are reported by Stewart, Bell and Roehlke (4). This conflict emphasizes the lack of full understanding of the nature and functions of the Leydig cells.

The normal or eunuchoid appearance is not what would be expected in a patient with testes showing hyperplasia of cells supposedly capable of producing an androgenic substance (testosterone), which is credited with the development of the male secondary sex characteristics. Thus it appears that the hyperplastic cells are nonfunctional. The possibility of a secretion from the seminiferous tubules must be considered.

An interesting corollary to the findings in this case is reported by Rasmussen (5). Seven patients with basophilism (not pituitary adenomas) were examined with special reference to the adrenals. Three showed change suggestive of cortical adenomas, two had cortical adenomas and two had pronounced hypertrophy of histologically normal cortices.

Our patient shows both the basophilism and the adrenal cortical adenoma. The relationship of these changes to the interstitial cell hyperplasia is, at present, speculative (6).

SUMMARY

A case of unusual hyperplasia of the interstitial cells of the testis is presented.

Other interesting endocrinologic features of the case are presented, namely, a basophilic hyperplasia of the pituitary gland and an adenoma of the cortex of one adrenal gland.

Some of the questions posed by the various endocrine changes are discussed.

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NECROPSY STUDY OF A CASE OF TURNER'S SYNDROME

A CASE REPORT

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TURNER (1) in 1938 described a syndrome characterized by 1) arrest or great delay in growth and ossification (dwarfism), 2) great delay in sexual development (infantilism), and 3) congenital webbing of the neck and cubitus valgus.

CASE REPORT

In 1945 one of us (2) reported a case of this syndrome. The patient was a white female, 20 years old, with primary amenorrhea. Her father was an alcoholic. She was born prematurely. Her feeding was artificial and insufficient, and her somatic development had been retarded since birth. When she was 6 years old she suffered a cranial trauma with loss of consciousness. Two years later she had diphtheria. The serum with which she was treated caused serum sickness.

Physical Examination: The body measurements were those of a girl 8 years old. Her face had a peculiar aged appearance, and the somatic proportions were definitely eunuchoid. She had a congenital webbed neck, and neither mammary development nor sexual hair. The external genitalia resembled those of a girl 3 to 5 years old. The uterus was about the size of a hazelnut. In the upper extremities a pronounced cubitus valgus was noticed.

Roentgenograms of the skull, hands and ankles revealed a bone age between 10 and 14 years.

A vaginal smear showed pronounced estrogenic deficiency with complete absence of cornified cells. Urinary excretion of 17-ketosteroids in 24-hour urine specimens was, on the average, 3.5 mg. The basal metabolic rate was within normal limits.

We could not perform quantitative gonadotropic hormone assays, but we injected the patient's urine into a female rabbit, previously isolated for a month, according to the usual technique. This caused the appearance of a great number of primary follicles in the animal's ovaries. Thus an increased urinary excretion of gonadotropic hormone was demonstrated.

Following the therapy recommended by Albright et al. (3) and Hamblen (4), we treated the patient with stilbestrol and small doses of desiccated

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thyroid. She grew 1.5 cm., and her body weight increased 1.5 Kg. after three months of treatment.

An intercurrent attack of acute appendicitis warranted a laparotomy. The appendix and a small piece of the left ovary were removed. The patient died twenty hours after the operation in what appeared to be acute cardiovascular shock.

Autopsy Findings: The autopsy was done three and one-half hours after death. The body had an infantile appearance with scanty mammary and genital development. A surgical median scar was noted on the abdominal

FIG. 1. Brain.

wall. The appendix and a small piece of the left ovary had been removed (biopsy).

The positive autopsy findings were as follows:

1. The shortness of the neck was due to a congenital luxation of the atlanto-odontoid joint. The brain surface was normal grossly (Fig. 1).

2. The internal genitalia were infantile. The uterus measured 3.5 cm. (⅔ cervix, ⅓ fundus). The ovaries were fusiform bodies weighing barely 2.5 Gm. each.

3. Lymphoid tissue was abundant in the inguinal and axillary regions, mesentery, Waldeyer's ring and Peyer's patches.

4. There was marked lymphoid hyperplasia in the mucosa and submucosa of the appendix. This was evidently related to the acute symptoms of appendicitis (Fig. 2).

5. In all the organs there were signs of acute vascular shock.

Macroscopic and Microscopic Findings in Endocrine Glands: These findings are of particular interest since there appears to be no other detailed report of an identical case. The cases reported by Rössle and Wallart (5), Hegar (6), and Hegar and Freund (7), do not correspond exactly with this clinical entity.

The pituitary was normal grossly. We observed no regression, or alterations of the stroma or vascular system of the gland. The anterior lobe ap-

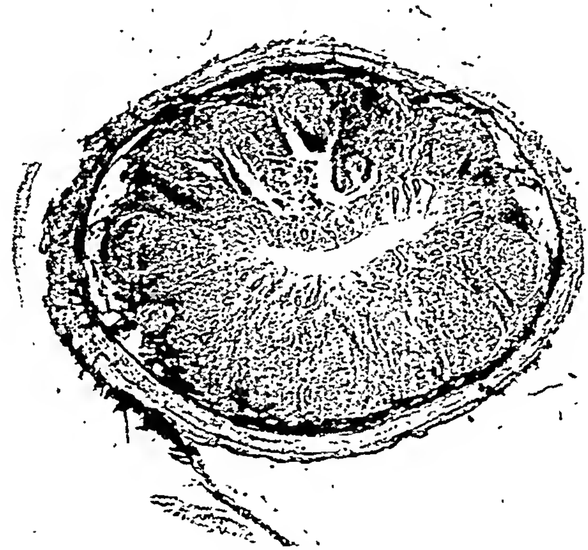

FIG. 2. Vermiform appendix. Hem-eos. ×12.

peared to consist of cellular cordons arranged in follicles. They formed small cavities containing acidophil colloid (Fig. 3). Acidophil and chromophobe cells were predominant, whereas the basophil cells were scanty (Fig. 4). There seemed to be an abnormally high proportion of acidophil cells, but no cell count was made to validate this impression. Proportions of the different cell types in the anterior lobe vary greatly, according to the different techniques used by different observers; hence we did not think it wise to draw any conclusions as to the functional status of the gland (8–13).

The thyroid was small. The follicles measured from 100 to 200 microns in diameter, the epithelial cells being low and the colloid abundant. There were several lymphoid follicles present (Fig. 5).

The pancreas was normal grossly. The islands of Langerhans were normal

microscopically, and alpha and beta cells could be easily distinguished (Fig. 6).

The suprarenals weighed 2.5 Gm. The ideal weight should be 11 Gm. according to the chronologic age of the patient and 7 Gm. according to the somatic age (14). Microscopically the general structure was intact, the

FIG. 7. Suprarenal cortex. Hem.-eos. ×200.

medullary part being well developed (Fig. 7). The cortical section appeared hypoplastic, especially in the glomerular zone.

The thymus weighed 35 Gm. which was excessive for the patient's chronologic and somatic age. The histologic appearance was typically infantile with no tendency to fatty degeneration, and the parenchyma was well developed, the cortical zone being predominant (Fig. 8).

The ovaries were also infantile; their weight was 2.5 Gm. In the cortical part many primary follicles were seen; in the central part well developed

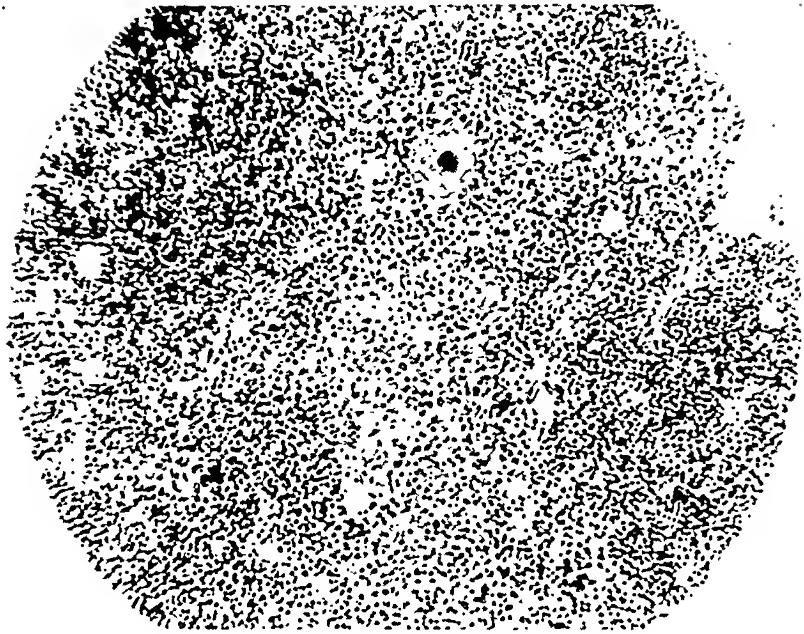

FIG. 8. Thymus. Hem.-eos. ×30.

FIG. 9. Ovary. Hem.-eos. ×15.

follicles with a tendency to cystic degeneration were present. No rupture or luteal changes were noted. The absolute lack of growing and intermediate follicles was striking (Figs. 9, 10).

The endometrium of the uterus was also infantile in character, 150 microns high, with ramified glands of high epithelium and acidophil cytoplasm. The stroma was latent in development, with very few cells (Fig. 11)'

FIG. 10. Ovary. Hem.-eos. ×5.

FIG. 11. Uterine endometrium. Hem.-eos. ×45.

DISCUSSION

These anatomic findings supported our diagnosis. To explain the pathogenesis of the symptoms and signs on the basis of an intrinsic ovarian failure alone does not seem logical to us. Hamblen (4) also rejects this explanation. We suggest that the cause originates outside the ovary and is a congenital developmental defect affecting principally the ovaries and suprarenals. On this basis the retarded development of the patient continued, ending in a dwarfism with eunuchoid proportions.

The large thymus and general lymphoid hyperplasia may be related to the hypoplasia of the adrenal cortex. This hypoplasia may bear some relation to the inability of the patient to react adequately to the stress of the appendectomy.

SUMMARY

a. The necropsy and microscopic findings in a case of Turner's syndrome are reported.

b. The anterior pituitary gland was normal grossly. A congenital luxation of the atlanto-odontoid joint, and ovarian and suprarenal hypoplasia were noted. These findings seem adequate to explain the peculiar characteristics of the dwarfism and infantilism.

c. It is suggested that a congenital unknown cause interfered with normal embryologic morphogenesis, affecting principally the gonads and suprarenals, and in a lesser degree the rest of the endocrine system.

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INDUCTION OF UTERINE BLEEDING IN AMENORRHEA WITH A SINGLE INJECTION OF PRECIPITATES OF ESTRONE AND PROGESTERONE

BERNHARD ZONDEK AND SAMUEL ROZIN

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HITHERTO it has generally been thought that to induce bleeding, the uterine mucosa had first to be stimulated to proliferation by estrogenic hormone and that the secretory phase had subsequently to be induced by progesterone. The doses required for this effect were from 30 to 40 mg. of estradiol benzoate and from 25 to 40 mg. of progesterone. This course of treatment lasted for twenty-four days, and required eight injections of 5 mg. each of estradiol benzoate over a period of twenty days followed by daily injections of 5 mg. of progesterone for five days. In many cases bleeding set in a few days after the injections had been discontinued. With this form of therapy the patient received thirteen injections in one month. We think that such a course should be avoided for the following reasons: 1) The prolonged treatment, which makes the patient dependent on the physician for a long period, intensifies the inferiority complex from which the amenorrheic woman already suffers. 2) The 40 mg. of estradiol benzoate necessary for the proliferation of the uterine mucosa exerts an inhibiting effect on the gonadotropic function of the anterior lobe of the pituitary (1), a result which is unconditionally to be avoided in amenorrheic women. If such treatment be continued for several months, the large doses of estrogens cause more harm than benefit, since, although uterine bleeding is thereby induced by local action on the uterine mucosa, at the same time spontaneous regeneration of the gonadotropic function of the pituitary gland is prevented.

In 1942, Zondek (2) recommended a simplified treatment of amenorrhea. The formerly held view that bleeding can be induced only with the aid of progesterone acting on a uterine mucosa previously brought to full proliferation by estrogens, was shown to be incorrect. In normally menstruating women it was found that the administration of 50 mg. of progesterone on the fifth to the tenth day of the cycle brought on a bleeding in the intermenstruum lasting from four to five days ("intracyclic bleeding"), which was considered by the patients to be similar to a normal menstruation

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(3, 4). Anatomic examination demonstrated that such bleedings had their origin in a typical, thin, intermenstrual mucosa, containing few glands and no glycogen. It was thereby shown that progesterone alone is capable of inducing a bleeding even in an undeveloped mucosa and that the proliferation induced by estrogens is not an essential prerequisite in the bleeding mechanism.

These observations were then utilized in the treatment of amenorrhea. It was surprising to find that it required only two to five days of treatment, with a total dose of 50 mg. of progesterone, to induce bleeding in amenorrheic women. The dose of progesterone could be reduced to 25 mg., if 2.5 mg. of estradiol benzoate were administered concurrently, in cases of amenorrhea which were of not more than two years' duration. It should again be emphasized that in this simplified method, bleeding originates in a thin mucosa, only slightly proliferated. A minimal estrogen stimulus seems, however, to be necessary in primary amenorrhea since progesterone

TABLE 1. SIMPLIFIED TREATMENT OF AMENORRHEA WITH HORMONE SOLUTIONS

Days of Treatment	Estradiol Benzoate, mg.	Progesterone, mg.
Primary Amenorrhea		
5	2.5–5	50
Secondary Amenorrhea		
Of more than 2 years' duration		
5	—	50
2	—	50
Of less than 2 years' duration		
2	2.5	25

alone is incapable of inducing bleeding, and from 2.5 to 5 mg. of estrogens must be administered together with the 50 mg. of progesterone in the 5-day treatment of such cases. Results with our simplified method are summarized in Table 1. Our experience with this method of treatment of amenorrhea has been corroborated by Berlind (5), Finkler (6), Rakoff (7), and others.

METHOD FOR SINGLE-INJECTION TREATMENT

The following data demonstrate that the treatment of amenorrhea may be further simplified and that a single injection may be substituted for the two to five injections hitherto required to induce bleeding. This can be achieved if estrone and progesterone be injected not as a solution but as

very minute crystals (estrone) or in amorphous form (progesterone). The mechanism of action will be reported in another paper, and only brief mention will be made of this matter in this article. Estrone and progesterone are dissolved in an organic solvent miscible with water (a mixture of various homologous ester derivatives of α-β-dichlorhydrin), and the hormones are precipitated from this solution in the syringe by the addition of saline solution.[1] The crystals are precipitated in so fine a form that they do not occlude the lumens of the needles used for intragluteal injections. Constancy of size of the precipitated crystals is insured by an optimum proportion between the solution of the hormones and the precipitator. The fact that the size and form of the hormone crystals have undergone a change as a result of precipitation is shown by the following observations: a) crystallized estrone, which generally occurs in rhombic form, is precipitated by this process in the form of delicate, ice-fern-like needles; b) progesterone, generally in the form of prisms and needles, is precipitated in amorphous form, and crystals begin to form again only after a considerable length of time; c) the mixture of estrone and progesterone is also precipitated in amorphous form, and from this suspension progesterone crystals in particular are redeposited after the lapse of a certain period. Twenty-four hours after the precipitates are injected subcutaneously into rats few crystals of estrone can be found, whereas many more crystals of progesterone, recrystallized from the injected mixture, are observed. If crystals of estrone and progesterone in their original form are suspended in water, in which form the crystals are considerably larger than in our preparations, bleeding cannot be induced in amenorrheic women by a single injection. It follows that the size of the crystals is of decisive significance.

RESULTS

We have excluded from our series those cases in which amenorrhea was but a symptom of general disease, for example, tuberculosis, diabetes and avitaminosis. In the patients treated, amenorrhea had either lasted an average of four years (secondary amenorrhea) or the women had never menstruated (primary amenorrhea). In all cases there was a distinct disturbance in the development of the genital organs, atrophy of the vagina and especially a definite hypoplasia of the uterus, which was reduced to as little as 3 cm. in length. The fact that production of estrogenic hormone was diminished or nonexistent was proved by increased excretion of follicle-

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[1] We are indebted to "Teva," Middle East Pharmaceutical & Chemical Works, Ltd., Jerusalem, for supplying us with the preparation (Ambo-Pelletoids).

stimulating hormone in the urine (8) in all the patients with primary amenorrhea and in some of the patients with secondary amenorrhea (100 M.U. or more per liter of urine). There was no development of the uterine mucosa, as investigated by the progesterone test (3), in all patients with primary and in some with secondary amenorrhea; that is, 50 mg. of progesterone did not induce bleeding, which resulted only after the administration of additional estrogenic hormone. In all our patients, strip curettage showed the uterine mucosa to be underdeveloped, the mucosa being thin and containing a few glands only in the postmenstrual phase. The vaginal smear showed diminished or absent estrogenic activity. There were a few patients with secondary amenorrhea showing signs of mild hypothyroidism combined with adiposity, who responded promptly with bleeding during our treatment.

A detailed report will be published elsewhere.

Primary amenorrhea

We treated 5 patients with primary amenorrhea.

Patients 1 and 2 each received 5 mg. of estrone and 50 mg. of progesterone in a single injection, and both reacted after nine and ten day intervals, respectively, with bleeding lasting for four days.

Patient 3 did not react. This patient was a 37-year-old woman, who had already received several courses of hormone treatment of as much as twenty days' duration without success.

Patient 4 received three treatments at monthly intervals, each treatment consisting of 5 mg. of estrone and 50 mg. of progesterone. The patient responded every month with bleeding ten days after each injection. Although she received no treatment the fourth month, a spontaneous bleeding occurred after an interval of twenty-four days. Since this time the patient has menstruated spontaneously four times, at intervals of twenty-five to twenty-eight days. The fact that *spontaneous* bleeding was observed in a case of primary amenorrhea suggests that our treatment may not only constitute substitution therapy but may actually serve as a stimulus of the gonadotropic function of the anterior pituitary lobe, which regulates the bleeding mechanism. Similar results were obtained in the treatment of secondary amenorrhea, as reported in the next section.

Patient 5 was initially treated with 8 injections of 40,000 U of estradiol benzoate together with 5 mg. of progesterone over a period of twenty-four days, but bleeding could not be induced thereby. This patient was a 20-year-old girl in whom secondary sex characteristics were totally absent. After rectal examination, it was still doubtful whether or not a uterus was present, but the introduction of a speculum revealed a cervix the size of a pea. This observation justified the assumption that a minute uterus did

exist. This patient received 10 mg. of estrone together with 50 mg. of progesterone in a single injection. Ten days later bleeding began, and continued for a six-day period. After a second treatment with 5 mg. of estrone together with 50 mg. of progesterone, bleeding occurred after an interval of seven days.

Secondary amenorrhea

We have found in cases of secondary amenorrhea that, in order to induce bleeding, larger doses of progesterone must be given in the first treatment but that these doses may be reduced in the second and third treatments. This observation seems to be of interest, inasmuch as it shows that the body may have been rendered more sensitive to the action of the hormone.

A patient with secondary amenorrhea responds with bleeding after the injection of 5 mg. of estrone and 50 mg. of progesterone or, in milder cases, of 50 mg. of progesterone alone. The progesterone dose may then be reduced by half in the second and third treatments, when 5 mg. of estrone and 25 mg. of progesterone suffice. According to our experience to date, we recommend from three to four treatments at monthly intervals in order to imitate the normal cycle. After such a course 5 of our 29 patients treated for secondary amenorrhea have to date menstruated spontaneously. One of them menstruated three times, and 4 of them twice each, after intervals of twenty-two and twenty-seven days, respectively. Spontaneous bleeding in a case of primary amenorrhea has been reported in the previous section. The fact that cyclic estrone-progesterone treatment is capable of inducing spontaneous bleeding in subsequent months supports the view that such treatment may stimulate the gonadotropic function of the anterior pituitary lobe, so that the machinery of sexual function is again set in motion. It is noteworthy that G. V. Smith (9) recommends cyclic treatment as the method of choice to re-establish a normal interrelationship between the pituitary gland and the ovaries.

Our single-injection method of treatment is satisfactory even in severe cases of secondary amenorrhea, as may be seen from the fact that 3 patients, having amenorrhea for nine years, regularly responded with bleedings lasting from three to five days during twelve treatments. Bleeding was induced five times in a woman who had amenorrhea for five years; and 3 patients who had amenorrhea for four years were successfully treated. In addition, among 10 women who had amenorrhea for three years, 8 responded with bleedings. Since the average duration of amenorrhea in our cases was four years, it is clear that we did not select mild cases. Only 3 patients who had amenorrhea lasting for less than two years received this treatment.

SUMMARY

To date 34 patients with amenorrhea have been treated.

a) Five patients with primary amenorrhea received eight treatments, of which seven (87.5 per cent) were successful.

b) Twenty-nine patients with secondary amenorrhea received forty-three treatments, of which forty (93 per cent) were successful. Bleeding began from seven to ten days after injection and lasted for an average of four days.

CONCLUSIONS

1) A new treatment of amenorrhea with a single injection of estrone and progesterone has been described. Estrone and progesterone, dissolved in an organic solvent, are precipitated in the syringe by the addition of saline solution, the suspension being injected intragluteally.

2) The doses of hormone required in the single-injection treatment of primary amenorrhea are 5 to 10 mg. of estrone and 50 mg. of progesterone.

3) The doses of hormone required in the single-injection treatment of secondary amenorrhea are as follows: In the first treatment 5 mg. of estrone and 50 mg. of progesterone should be administered. In the subsequent treatments the dose of estrone remains the same, but the dose of progesterone may be reduced to 25 mg.; or 50 mg. of progesterone without estrone may be used. This decreased requirement may indicate that the body has been rendered more sensitive to the hormones.

4) Three to four treatments at intervals of four weeks should be given, and spontaneous bleeding should then be awaited. We observed spontaneous cyclic bleeding in 5 patients with secondary amenorrhea and in 1 patient with primary amenorrhea. This leads us to assume that the treatment not only constitutes a substitution therapy, but perhaps actually provides a stimulation of the gonadotropic mechanism in the anterior lobe of the pituitary.

5) Advantages of the suggested method of treatment are: a) Only one injection is required, a fact which is of psychological importance to the patient. b) The anterior lobe of the pituitary is not inhibited by the small dose of estrogenic hormone administered (5 mg.). c) The intragluteal injections are well tolerated; secondary reactions, such as pain, have been observed in only a few cases.

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THE THIRTIETH ANNUAL MEETING OF THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS

GENERAL INFORMATION

Headquarters: Red Lacquer Room of the Palmer House, Chicago, Illinois.

Registration: Everyone attending the meetings is requested to register. A fee of $1.00 will be charged non-members of the Association. Membership cards should be presented when registering.

The Scientific Sessions: The Scientific sessions will be held in the Red Lacquer Room of the Palmer House and programs will begin promptly on schedule. Papers presented at all meetings are planned for ten minutes and owing to the heavy schedule must be kept within this limit. Manuscripts of all papers should be submitted to the presiding officer or Secretary-Treasurer at the end of the presentation.

Annual Dinner: The Annual Dinner of the Association will be held on Friday evening, June 18th, at 7:30 o'clock in the Red Lacquer Room of the Palmer House, preceded by cocktails at 6:30 o'clock. Secure tickets at time of registration.

Council Meetings: There will be a meeting of the Council on Thursday afternoon, June 17th, at 2:00 o'clock, and a luncheon meeting on Friday June 18th.

Business Meeting: The Annual Business Meeting of the Association and Election of Officers will be held at 4:30 P.M., June 19th, in the Red Lacquer Room of the Palmer House.

Local Arrangements: Dr. Willard O. Thompson, 700 N. Michigan Ave., Chicago, Illinois, is in charge of the local arrangements for the meetings.

Secretary-Treasurer: Henry H. Turner, 1200 North Walker Street, Oklahoma City 3, Oklahoma.

PROGRAM

FRIDAY, JUNE 18, 1948

8:30 a.m. Registration

I. 9:30 a.m. Red Lacquer Room

J. S. L. Browne, *presiding*

1. PSEUDO-GLANDULAR DISTURBANCES.
   by Hugo R. Rony
2. SYNDROME OF CRYPTORCHIDISM, HEART DISEASE AND NEVOID DERMATOSIS.
   by S. J. Glass
3. CONSTITUTIONAL PRECOCIOUS PUBERTY CONTROLLED BY ANDROGEN THERAPY.
   by S. Charles Freed and Minnie Goldberg
4. A STUDY OF THE BIOLOGICAL ACTIVITY AND THE MAGNITUDE OF ENDOGENOUS ANDROGEN PRODUCTION IN A CASE OF ANDRENOGENITAL SYNDROME.
   by Anne C. Carter and Ephraim Shorr
5. PSEUDOHERMAPHRODISM. EARLY AND LATE RECOGNITION.
   by M. James Whitelaw
6. CLINICAL, LABORATORY, OPERATIVE AND POSTMORTEM OBSERVATIONS IN INFANTS AND CHILDREN WITH MULTIPLE CONGENITAL MALFORMATIONS (TURNER'S SYNDROME, OVARIAN AGENESIS AND RELATED COMBINATIONS).
   by Frank L. Plachte (introduced by Henry H. Turner)
7. A SYNDROME CHARACTERIZED BY HYPERCALCEMIA, CALCINOSIS, AND RENAL INSUFFICIENCY FOLLOWING PROLONGED INTAKE OF CALCIUM AND ALKALI.
   by Charles H. Burnett, Robert R. Commons (by invitation), Fuller Albright and John E. Howard
8. HYPOPARATHYROIDISM, WITH MENTAL TROUBLES AND ECTODERMAL DISORDERS.
   by Manuel Villaverde
9. TREATMENT OF FAR ADVANCED INOPERABLE CARCINOMA OF THE BREAST WITH ESTROGENS AND ANDROGENS.
   by Samuel G. Taylor, III, Danely Slaughter (by invitation) and Frederick W. Preston (by invitation)
10. HORMONAL FACTORS INVOLVED IN THE REGULATION OF BODY TEMPERATURE DURING MENSTRUAL CYCLE AND PREGNANCY.
    by Charles L. Buxton and William B. Atkinson
11. THE EFFECTS OF CERTAIN STEROIDS—INTRAMUSCULAR AND SUBLINGUAL—ON THE BASAL BODY TEMPERATURE OF THE ADULT HUMAN MALE.
    by Robert M. Perlman

II. 2:00 p.m. Red Lacquer Room

C. N. H. LONG, *presiding*

12. A SIMPLIFIED HYPOPHYSECTOMIZED RAT ADRENAL ASCORBIC ACID BIOASSAY METHOD FOR ADRENOCORTICOTROPHIN (A.C.T.H.); SPECIFICITY AND APPLICATION TO PREPARATIVE PROBLEMS.
    by Paul L. Munson, Alfred G. Barry, Jr. (by invitation), and F. C. Koch
13. CONTENT OF ADRENOCORTICOTROPHIC HORMONE (A.C.T.H.) IN THE RAT PITUITARY UNDER OPTIMAL AND STRESSFUL ENVIRONMENTAL CONDITIONS.
    by George Sayers, Marshal Merkin (by invitation) and J. N. Tortoreto (by invitation).
14. THE ACTIVATION OF THE ADRENAL CORTEX BY INSULIN HYPOGLYCEMIA.
    by H. Gershberg (by invitation) and C. N. H. Long.
15. INFLUENCE OF ADRENOTROPHIC HORMONE ON SODIUM EXCRETION IN HYPOPHYSECTOMIZED RATS.
    by Betty L. Rubin (by invitation) and Ralph I. Dorfman

16. Factors Influencing the Corticotrophin Production of the Anterior Pituitary.
    by Hans Selye
17. The Use of Andrenocorticotrophin as a Test of Adrenal Cortical Reserve.
    by George W. Thorn, Peter H. Forsham (by invitation), Lillian Recant (by invitation) and A. Gorman Hills (by invitation)
18. Observations on the Pituitary-Adrenal Response Following Epinephrine Infusion in Man.
    by Lillian Recant (by invitation), Peter H. Forsham (by invitation) and George W. Thorn
19. Fate and Metabolic Action of Intravenously Administered Adrenocorticotrophic Hormone (A.C.T.H.).
    by Thomas W. Burns (by invitation), George Sayers, Frank H. Tyler (by invitation), B. V. Jager (by invitation), T. B. Schwartz (by invitation), Emil L. Smith (by invitation) and L. T. Samuels
20. Metabolic Changes Following the Administration of Pituitary Adrenocorticotrophic Hormone (A.C.T.H.) to Normal Humans.
    by H. T. McAlpine (by invitation), E. H. Venning, L. Johnson (by invitation), V. Schenker (by invitation), M. M. Hoffman and J. S. L. Browne
21. The Effect of Adrenocorticotrophin on Antibody Levels in Normal Human Subjects.
    by P. H. Herbert and J. A. de Bries (introduced by J. S. L. Browne)
22. A Comparison of the Effect on Bone Formation of the Hyperadrenocorticism of Cushing's Syndrome with that Induced by Adrenocorticotrophic Hormone (A.C.T.H.).
    by Frederic C. Bartter (by invitation), Anne P. Forbes and Fuller Albright
23. Adrenal Cortical Unresponsiveness in Patients with Gastric Cancer.
    by Edward C. Reifenstein, Jr., N. F. Young (by invitation), Aurelia Potor (by invitation), Benedict Duffy (by invitation) and F. Homburger (by invitation)
24. The Excretion of Adrenal Metabolites in Human Urine.
    by Konrad Dobriner, Seymour Lieberman (by invitation) and C. P. Rhoads (by invitation)

III. **Annual Dinner, Friday, June 18.**

7:30 p.m.—Red Lacquer Room, Palmer House

Presentation of E. R. Squibb and Sons Award for 1948
Presentation of Ciba Award for 1948
Presentation of Ayerst, McKenna and Harrison Fellowship for 1948.
by Warren O. Nelson, Chairman of the Committee on Awards 1947–48
President's Address: C. N. H. Long, Yale University

SATURDAY, JUNE 19, 1948

IV. **9:00 a.m. Red Lacquer Room**

R. G. Hoskins, *presiding*

25. Preparation of Crystalline Growth Hormone.

by Jacob B. Fishman (by invitation), Alfred E. Wilhelmi (by invitation) and Jane A. Russell

26. The Influence of Purified Growth Hormone on Fasting Metabolism.
by Clara M. Szego and Abraham White
27. Unpredictable Effects of Growth Hormone Preparations on Nitrogen Storage.
by Paul Bartlett (by invitation) and Oliver H. Gaebler
28. Studies in Growth. I. The Effects of Androgen in Gigantism and Acromegaly.
by Laurence W. Kinsell, George D. Michaels (by invitation), Choh Hao Li (by invitation) and William E. Larsen (by invitation)
29. The Effect of Iodine Injections on Energy Metabolism and Plasma Protein-Bound Iodine of Rats.
by S. B. Barker and H. J. Lipner (by invitation)
30. The Effect of Pituitary and Non-Pituitary Gland Factors on the Formation of Intracellular Colloid Droplets in the Thyroid Gland in Vivo and in Vitro.
by Samuel Dvoskin
31. Inactivation of the Exophthalmic, Thyrotropic and Ketogenic Principles of Anterior Pituitary Extract by Iodination.
by William McK. Jefferies
32. Newer Methods of Antagonizing Hyperthyroidism.
by Robert H. Williams, Rene F. Tagnon (by invitation), Herbert Jaffe, (by invitation), Beverly T. Towery (by invitation) and Walter F. Rogers (by invitation)
33. The Use of Radioactive Iodine (I 131) in the Study of Normal and Disordered Thyroid Function in Man.
by Sidney C. Werner and Edith Quimby (by invitation)
34. The Effect of Thyroid Stimulating Hormone on the Function of Human Normal and Malignant Thyroid Tissue.
by J. B. Trunnell (by invitation), R. W. Rawson, L. D. Marinelli (by invitation) and Ruth Hill (by invitation)
35. The Relation Between Infant Birthweight and Subsequent Development of Maternal Diabetes Mellitus.
by Joseph P. Kriss and Palmer H. Futcher (introduced by Cyril M. MacBryde)

V. 2:00 p.m. Red Lacquer Room

A. T. Kenyon, *presiding*

36. Absorption and Excretion of Chorionic Gonadotrophin When Administered Intramuscularly to Women.
by J. T. Bradbury and Willis E. Brown
37. The Renal Clearance of Chorionic Gonadotrophic Hormone in Pregnancy and in Neoplasm of the Testis.
by C. F. Gastineau (by invitation), A. Albert and L. M. Randall (by invitation)

38. THE METABOLIC RESPONSE TO CHORIONIC GONADOTROPHIN IN YOUNG MEN.
by Kathryn Knowlton (by invitation) and Allan T. Kenyon
39. BLOOD GONADOTROPHIN STUDIES DURING PREGNANCY IN RELATION TO THE FETAL SEX.
by H. E. Nieburgs and Robert B. Greenblatt
40. ON THE PRINCIPAL ESTROGENIC CONSTITUENTS OF THE URINE OF THE STALLION.
by Louis Levin
41. MECHANISM OF INACTIVATION OF α-ESTRADIOL BY RAT LIVER IN VITRO.
by R. H. deMeio (by invitation), A. E. Rakoff, A. Cantarow and K. E. Paschkis
42. COZYMASE IN THE HEPATIC INACTIVATION OF α-ESTRADIOL.
by Richard L. Coppedge (by invitation), Albert Segaloff, Herbert Sarett (by invitation) and Aaron Altshul (by invitation)
43. INTERFERENCE WITH ESTROGEN-INDUCED GROWTH IN THE FEMALE GENITAL TRACT BY FOLIC ACID.
by Roy Hertz
44. THE RELATION OF FOLIC ACID TO THE ACTION OF ESTROGENS.
by Irene T. Kline (by invitation) and Ralph I. Dorfman
45. FLUORESCENT PHENOMENA OF THE VULVA ASSOCIATED WITH SEX HORMONE METABOLISM.
by M. Sydney Margolese
46. TESTICULAR DEFICIENCY: A CLINICAL AND PATHOLOGICAL STUDY.
by R. Palmer Howard, Ronald C. Sniffen (by invitation) and Fred A. Simmons
47. A COMPARISON OF THE EFFECT OF VARIOUS ANDROGENS ON THE TEMPORAL MUSCLE AND ORGANS OF THE CASTRATED MALE GUINEA PIG.
by Charles D. Kochakian and Jane Harrison Humm (by invitation)

VI. Annual Business Meeting

5:00 P.M. Red Lacquer Room

Papers to be Read by Title

48. THE USE OF WHOLE ADRENAL CORTICAL EXTRACT IN EXPERIMENTAL INFECTIONS.
by Erwin P. Vollmer, James D. Gillmore (by invitation), Leo Cravitz (by invitation) and J. E. Samsell (by invitation).
49. THE WORK PERFORMANCE OF ADRENALECTOMIZED RATS GIVEN CONTINUOUS INTRAVENOUS INFUSIONS OF GLUCOSE.
by Dwight J. Ingle and James E. Nezamis (by invitation)
50. SUBLINGUAL ADMINISTRATION OF DESOXYCORTICOSTERONE ACETATE IN THE TREAMENT OF ADDISON'S DISEASE.
by Evelyn Anderson, Lawrence W. Kinsell, Troy C. Daniels (by invitation) and Edward Henderson
51. EXCRETION OF ADRENAL METABOLITES FOLLOWING THE ADMINISTRATION OF ADRENOCORTICOTROPHIC HORMONE TO NORMAL HUMAN SUBJECTS.
by Eleanor H. Venning, V. E. Kazmin (by invitation), Miriam Ripstein (by invitation), H. T. McAlpine (by invitation) and M. M. Hoffman

52. THE EFFECT OF 11-DEHYDROCORTICOSTERONE ON FECAL FAT EXCRETION.
by Grace E. Bergner (by invitation), Roger A. Lewis (by invitation), Frances W. Stout (by invitation), George W. Thorn and Kendall Emerson, Jr.
53. AN ESTIMATION OF THE QUANTITY OF 11-17-OXYSTEROID EXCRETION BY THE HUMAN ADRENAL STIMULATED BY ACTH.
by A. Gorman Hills (by invitation) and George W. Thorn
54. ISOLATION OF URINARY STEROIDS FROM A PATIENT WITH APPARENT ADRENAL INVOLVEMENT.
by A. M. Miller (by invitation) and Ralph I. Dorfman
55. THE EFFECT OF ADRENALECTOMY AND DESOXYCORTICOSTERONE ACETATE ADMINISTRATION UPON THE ECG RESPONSE OF THE RAT TO CARDIAC GLYCOSIDES.
by Herbert S. Kupperman, Joseph G. Benton (by invitation) and Arthur C. DeGraff (by invitation)
56. ACTIVATION OF THE ADRENAL CORTEX IN HUMAN SUBJECTS FOLLOWING ELECTROCONVULSIVE THERAPY (E.C.T.) AND PSYCHOMOTOR STRESS.
by R. A. Cleghorn and A. J. Goodman (by invitation), B. F. Graham, M. H. Jones and N. K. Rublee
57. EFFECT OF ADRENAL CORTICAL COMPOUNDS ON ELECTROLYTE METABOLISM OF A PATIENT WITH ADDISON'S DISEASE DURING HIGH SODIUM CHLORIDE INTAKE.
by Aurelia Potor (by invitation), Nelson F. Young (by invitation), F. Homburger (by invitation) and Edward C. Reifenstein, Jr.
58. NITROGENSPARING (PROTEIN-ANABOLIC) ACTION OF THYROID HORMONE.
by J. Rupp (by invitation) and K. E. Paschkis
59. THIOURACIL EFFECT ON PLASMA AND LIVER PROTEIN CONCENTRATIONS.
by James H. Leathem
60. RADIOIODINE UPTAKE BY THE THYROID AS AN AID IN DIFFERENTIAL DIAGNOSIS.
by S. M. Seidlin, E. Oshry (by invitation), I. Rossman (by invitation) and L. Leiter (by invitation)
61. THYROID UPTAKE OF RADIOACTIVE IODINE IN THE NORMAL AND HYPOMETABOLIC HUMAN.
by Martin Perlmutter (by invitation) and Peter H. Forsham (by invitation)
62. CELLULAR INVOLUTION IN THE THYROID.
by Nathan B. Friedman
63. METHYL THIOURACIL IN THE TREATMENT OF THYROTOXICOSIS.
by Grosvenor W. Bissell, John M. Benny (by invitation), Victor Totah (by invitation) and Florence Gilbert (by invitation)
64. MODIFICATION OF THE ESTRUAL CYCLE OF THE EWE BY THE USE OF PROGESTERONE; THE EFFECT UPON SUBSEQUENT OVULATION RATE AND FERTILITY OF OVA.
by R. H. Dutt (by invitation) and L. E. Casida
65. EFFECTS OF VARIOUS ESTROGENIC PREPARATIONS ON THE VAGINAL MUCOSA.
by Mildred Vogel (by invitation), Thomas H. McGavack and Joseph Mellow (by invitation)
66. THE USE OF THE VAGINAL SMEAR IN THE ASSAY OF ESTROGENS GIVEN ORALLY OR INTRAMUSCULARLY.
by Willis E. Brown and J. T. Bradbury

67. The Similarity of Estrogenic Effect in Premenstrual Tension, Menstrual Anomalies, Chronic Cystic Mastitis and Cancer of the Breast.
by Joseph H. Morton
68. Hyperestrogenism Treated with Lactogenic Hormone (Prolactin).
by Manuel Villaverde
69. The Factor of Rhythm in Experimental Menstruation.
by Doris H. Phelps
70. Hormonal Pellets in the Management of the Menopausal Syndrome.
by Robert B. Greenblatt and Roland R. Suran (by invitation)
71. The Effect of Hypophysectomy on the Ovulability of the Ovarian Follicle of the Domestic Hen.
by Irving Rothchild and R. M. Fraps
72. Prognostic Value of Pregnanediol Excretion in Threatened Abortion with Special Reference to the Effects of Diethylstilbestrol.
by A. R. Abarbanel
73. Further Studies on the Endometrial Cups of the Pregnant Mare.
by H. H. Cole and G. H. Hart
74. Inactivation of Posterior Pituitary Antidiuretic Hormone of the Liver.
by W. J. Eversole, J. H. Birnie (by invitation) and Robert Gaunt
75. The Estimation of Dehydroisoandrosterone and Related Compounds in Human Urine by a Modification of the Pettenkoffer Reaction.
by Richard L. Landau and Kathleen Lugibihl (by invitation)
76. Clinical Evaluation for 17-Ketosteroids by the Rapid Method.
by T. H. McGavack, S. Kenigsberg, A. M. Shearman and K. J. Drekter
77. The Application of Paper Partition Chromatography to Ketosteroids.
by Robert B. Burton (by invitation), Alejandro Zaffaroni (by invitation) and E. Henry Keutmann
78. A Rapid Modification of the Zimmermann Test for Ketosteroids.
by Sidney Pearson and Sylvester Giaccone (by invitation)
79. A Fluorometric Method for the Determination of Estrone and Estradiol in Human Urines.
by Joseph W. Jailer
80. A Clinical Bio-Assay for Chorionic Gonadotrophin.
by A. Albert
81. Specificity of a Colorimetric Method for Dehydroisoandrosterone in Urine Extracts.
by Paul L. Munson, Mary Ellen Jones (by invitation), Philip J. McCall (by invitation) and T. F. Gallagher (by invitation)
82. The Colorimetric Determination of Sodium and Its Application in the Study of Sodium and Chloride Balance.
by Joseph W. Goldzieher and Gilbert Stone (by invitation)
83. Depression of Lymphocyte Count After Orally Administered Glucose.
by Paul A. Marks (by invitation), Dorothy T. Marks (by invitation) and Joseph W. Jailer
84. Pitfalls in the Diagnosis of Diabetes.
by Bernard A. Watson
85. Electrolyte Balance Studies in the Uncontrolled and Controlled Diabetic State.

by Jonas Weissberg (by invitation), Thomas H. McGavack, A. M. Shearman (by invitation) and I. J. Drekter

86. The Role of the Endocrine Glands in Body Temperature Regulation.
by H. E. Nieburgs and Robert B. Greenblatt
87. The Lipogenic Activity of Propylthiouracil, Triphenylchloroethylene and Hexestrol in Chickens.
by R. George Jaap
88. The Influence of the Pituitary Gland on Blood Vessel Development. The Effect of Concentrated Pituitary Extract on the Isolated Kidney.
by Robert C. Moehlig and Louis Jaffe (by invitation)
89. Results of Prolonged Medical Treatment of Obesity with Diet Alone, Diet and Thyroid Preparations, and Diet and Amphetamine.
by David Adlersberg and Martin E. Mayer (by invitation)
90. The Urethral Smear in the Normal Human Male.
by Mildred T. Vogel (by invitation), Thomas H. McGavack and Henry Kammandel (by invitation)
91. The Specific Ovarian Hyperemic Inducing Effect of Luteinizing and Luteotrophic Hormones.
by Herbert S. Kupperman, W. H. McShan and Roland K. Meyer

PROGRAM OF THE 1948 LAURENTIAN HORMONE CONFERENCE

Forest Hills Hotel, Franconia, New Hampshire

Because of limited accommodations, attendance is by invitation, but the Committee on Arrangements will receive applications for membership until June 15, 1948. Applications should be addressed to: Dr. Gregory Pincus, Chairman, Committee on Arrangements, 222 Maple Avenue, Shrewsbury, Massachusetts.

I. Steroid Hormone Metabolism "in vivo" and "in vitro"

Some aspects of Progesterone Metabolism
Dr. G. F. Marrian, *University of Edinburgh*
Monday, Evening, September 13

Recent Developments in Our Knowledge of Estrogen Metabolism
Dr. R. D. H. Heard and Mrs. J. C. Saffran, *McGill University*
Tuesday Morning, September 14

The Metabolism of Androgens by Tissues
Dr. Leo T. Samuels, *University of Utah*
Tuesday Morning, September 14

The Metabolism of Estrogens with Particular Emphasis on Clinical Aspects of Physiology and Function of Ovarian Hormones
Dr. Albert Segaloff, *Alton Ochsner Medical Foundation*
Tuesday Evening, September 14

II. The Role of Hormones in Tissue and Body Metabolism

The Antihormone Problem in Endocrine Therapy
Dr. James H. Leathem, *Rutgers University*
Wednesday Morning, September 15

Integration of the Metabolic Effects of Adrenal Cortical, Thyroid and Growth Hormones
Dr. Abraham White, *University of California at Los Angeles*
Wednesday Morning, September 15

The Alterations in Metabolism Incident to Administration of Insulin, Adrenalin and Thyroid Substances, Studied with the Aid of Isotopes

Dr. DeWitt Stetten, Jr., *Harvard University Medical School*

Wednesday Evening, September 15

The Pancreas as the Guardian of the Liver

Dr. Charles H. Best, *University of Toronto*

Thursday Morning September 16

Metabolic Changes in Man Following Adrenal and Pituitary Hormone Therapy

Dr. George W. Thorn, *Harvard University Medical School*

Thursday Morning, September 16

III. Neurohumoral-Hypothalamic Relationships

Adrenal Function in Mental Disease

Dr. Gregory Pincus, Dr. Hudson Hoagland, Dr. Harry Freeman and Mr. Fred Elmadjian, *Worcester Foundation for Experimental Biology*

Thursday Evening, September 16

Manifestations of Altered Autonomic and Humoral Functions in Psychoneuroses

Dr. Robert A. Cleghorn, and Dr. B. F. Graham, *McGill University*

Friday Morning, September 17

Effects of Hypothalamic Lesions on Water and Energy Metabolism in the Rat

Dr. James A. F. Stevenson, *Yale University*

Friday Morning, September 17

IV. Thyroid Physiology and Function

Physiologic Reactions of the Thyroid Stimulating Hormone

Dr. Rulon W. Rawson and Dr. William L. Money, *Massachusetts General Hospital*

Friday Evening, September 17

The Metabolism of Iodine as Disclosed by the Use of Radioiodine (I131)

Dr. F. R. Keating, Jr., and Dr. Alexander Albert, *The Mayo Clinic*

Saturday Morning, September 18

Radioiodine as a Diagnostic and Therapeutic Tool in Clinical Medicine

Dr. S. M. Seidlin, *The Montefiore Hospital*

Saturday Morning, September 18

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CLINICAL
ENDOCRINOLOGY

VOLUME 8 JUNE, 1948 NUMBER 6

SPONTANEOUS AND EXPERIMENTALLY INDUCED UPTAKE OF RADIOACTIVE IODINE IN METASTASES FROM THYROID CARCINOMA: A PRELIMINARY REPORT*†

S. M. SEIDLIN, M.D., E. OSHRY, B.S., AND
A. A. YALOW, PH.D.

From the Medical Division and the Medical Physics Research Laboratory, Montefiore Hospital, New York, N. Y.

METASTATIC carcinoma of the thyroid can be successfully treated with radioiodine when the lesions concentrate this isotope (1). The usefulness of this method depends upon the percentage of cases in which such concentration can be demonstrated. A technique of inducing uptake of radioiodine in metastases that previously showed none would increase the usefulness of the method. Clinically we have been successful in inducing such uptake in several cases with subsequent amelioration of the disease.

In a recent publication by Marinelli *et al.* (2), it is reported that "approximately 15 per cent of thyroid cancers may be expected to accumulate radioactive iodine in some degree." This conclusion is based on findings in a selected group of 19 cases modified by an estimation of relative frequency of various types of thyroid carcinoma.

The present paper is a report on 14 unselected cases of metastatic thyroid carcinoma studied at Montefiore Hospital up to June 1947.

Received for publication March 18, 1948.

* Presented at the Twenty-ninth Annual Meeting of the Association for the Study of Internal Secretions, Atlantic City, New Jersey, June 7, 1947.

† This work was aided by grants from the Dazian Foundation for Medical Research and from Mr. and Mrs. M. Lincoln Schuster.

METHOD

Tracer doses ranging from 500 to 2000 microcuries of radioactive iodine (I^{131}) were administered orally to patients on nonrestricted diets. All urines were collected for at least the first forty-eight hours and studied for radioactive iodine content. Forty-eight hours after the dose was administered,

RADIOIODINE UPTAKE BY METASTASES FROM THYROID CARCINOMA

PATIENTS	GEIGER COUNTER ON ADMISSION	RADIOAUTOGRAPHS
R.A.	–	
W.B.	–	
A.B.	–	
B.B.	+	+
S.C.	–	
M.F.	–	
J.F.	–	
L.H.	+	+
L.L.	–	+
L.O.	+	+
M.S.	+	+
A.V.	–	
O.W.	–	
D.Y.	+	+
Totals: 14	5 + 36% 9 – 64%	6 + 43% 8 not done. 57%

FIG. 1

in vivo readings were taken with a Geiger-Mueller counter to determine the degree of localization of radioactive iodine in the thyroid gland and the metastases. The cases listed as positive for uptake in metastases as indicated by external Geiger counter measurements are those in which there was no doubt that the lesion contained a significantly higher concentration of radioiodine than a control area. The cases listed as negative are those in which it was not possible to demonstrate such a concentration.

In the later cases of the series, quantitative measurements were made to determine the percentage of the administered dose concentrated in the gland.

In cases submitted to biopsies radioautographic studies were carried

CASES ORIGINALLY "NEGATIVE" TREATED WITH T.S.H. OR I*

PATIENTS	TREATMENT	STATUS AFTER Rx
R.A.	I* (103 mc. I^{131} - 80% excreted in first 4 days)	Unknown, patient left hospital.
W.B.	T.S.H. (1 cc. Armours T.S.H. daily 4 - 5 weeks)	Negative
A.B.	T.S.H. (2 cc. Armours T.S.H. daily 17 times)	+
J.F.	I* (100 mc. I^{131} - 82% excreted in first 4 days)	+
L.L.	I* (10 mc. I^{131} ~ 70% excreted in first 4 days)	+

The other four negative cases were not treated in any way.

FIG. 2

out. A "large tracer" or a therapeutic dose of radioiodine was administered, usually forty-eight hours before the procedure. The tissue obtained at biopsy was imbedded in paraffin and sections cut, ten microns or less in thickness. The paraffin on at least 3 sections from each block was removed with xylol, an estimate of activity was obtained in a Geiger counter and the slides were exposed to a photographic emulsion for a variable period of time as determined from counter measurements. After the necessary exposure time, the slides were removed from the emulsion and stained and the films developed according to usual photographic technique.

RESULTS

As indicated in Figure 1, five out of the 14 cases showed uptake in at least one metastatic lesion the first time radioiodine was administered. Subsequently, radioautographs were made from tissue removed from six of the

14 patients. In all these cases the radioautograph showed definite concentration of radioactive iodine in the metastatic tissue. In one, external measurements had not indicated uptake, showing that the radioautographic technique, when feasible, is more sensitive than external measurements.

All the "positive" cases had previously had *a complete or nearly complete* thyroidectomy (B.B. and L.H. (3)) or a subtotal thyroidectomy followed by x-ray treatment. However, not all patients who had had partial thyroidectomy and x-ray treatment showed radioiodine uptake by metastases.

In some "negative" cases an attempt was made to induce uptake of radioiodine by pretreatment with thyrotropic hormone of the pituitary (T.S.H.) or by performing a "radiation thyroidectomy" through administration of a large dose of radioactive iodine. The results are shown in Figure 2.[1] Of the two cases treated with T.S.H. the results were positive in one and negative in the other.

Radiation thyroidectomies were attempted in 3 patients. One of these left the hospital too early for the results to be evaluated. In the other two, iodine uptake was induced in metastatic lesions. In one, J. F., there was a metastatic lesion in the skull which showed no uptake of I* at first. Neither a short series of injections of thyrotropic hormone nor a short course of thiouracil therapy caused any detectable I* uptake in this metastasis. Therefore, a 100 millicurie dose of I^{131} was administered. Six weeks later a tracer dose was given and Geiger counter readings showed definite uptake in the skull lesion. The other patient, L. L., originally showed no uptake in a lesion in the sternum. A 10 millicurie dose of I* was given. The next tracer dose about 2 months later showed uptake in this lesion.

Figures 3, 4, 5 and 6 are microphotographs of slides from the tumor tissue of patients listed in Figure 2 before any treatment was instituted. The last three are examples of highly differentiated thyroid tumors which did not concentrate radioiodine until stimulated by thyrotropic hormone, directly by injections of T.S.H. or indirectly by thyroidectomy.

In addition, in a "positive" case, M. S., the uptake of radioiodine in the tumor tissue was increased several-fold by a course of injections of T.S.H.

On each of 8 patients who had received radioiodine therapy, 1 to 5 curves of radioiodine concentration in the blood were taken. All of them were similar in shape to the one shown in Figure 7. However, after the first day, the radioiodine concentration in the one who had a "total" thyroid-

[1] The symbol I* used in this figure and throughout the article is the standard abbreviation for radioactive iodine.

FIG. 3. Patient W. B. Section of tumor shows highly anaplastic tissue with some areas of differentiation.

FIG. 4. Patient A. B. Section of tumor shows marked differentiation: definite follicle formation with colloid.

FIG. 5. Patient J. F. Section of tumor shows marked differentiation: follicle formation with colloid.

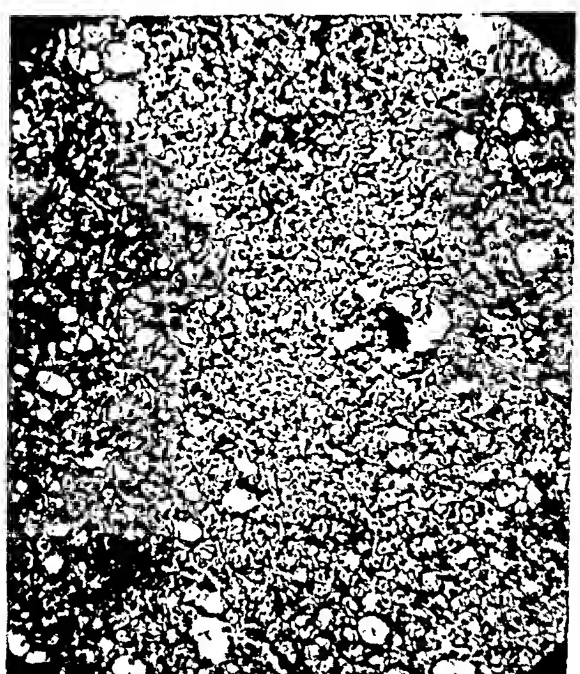

FIG. 6. Patient L. L. Section of tumor shows anaplastic areas as well as areas of varying degrees of differentiation.

ectomy was at a higher level than in any of the others. It appears that the level at which the curve flattens out may be an indication of the amount of normal thyroid tissue present in a given patient.

COMMENTS

In 1940, Seidlin (4) demonstrated the *inactivation* of *T.S.H.* by normal thyroid tissue, both in vivo and in vitro. About two years later the in vitro experiments were confirmed by Aub and his associates (5). Whatever

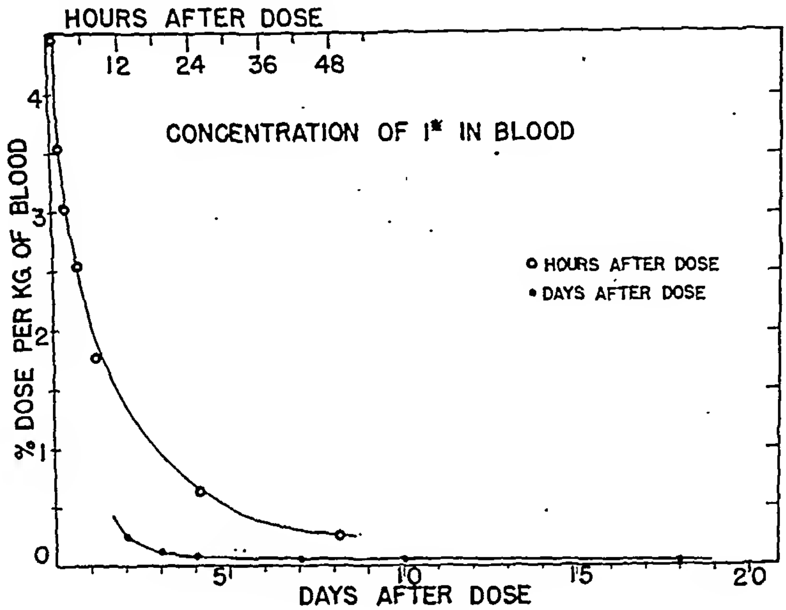

Fig. 7

the chemical reaction that takes place at the site of inactivation, the end result is stimulation of the thyroid tissue. This has been repeatedly demonstrated for *normal* thyroid tissue, both in vivo and in vitro.

Leiter, Seidlin, Marinelli and Baumann (3) have shown that at least in the two patients, B. B. and L. H., the metastases function in many respects like normal thyroid tissue. In our completely thyroidectomized patient (B. B.) intramuscular injections of thyrotropic factor were not followed by the appearance of T.S.H. in his urine. We felt justified in concluding that his metastatic thyroid carcinoma inactivated the T.S.H. (1).

In a patient who has both normal thyroid tissue and metastatic thyroid carcinoma, the administered I* goes primarily to the normal tissue. The normal thyroid also competes successfully for the T.S.H. in the blood, and consequently for the circulating or administered iodine. The above

two processes are separate reactions although the second accelerates the first. Thyroidectomy (surgical or radiation) enables the tumors to react with more T.S.H. In the process of inactivating the T.S.H. the tumor is likely to be stimulated to a higher level of functioning activity and to take up iodine (Fig. 8).

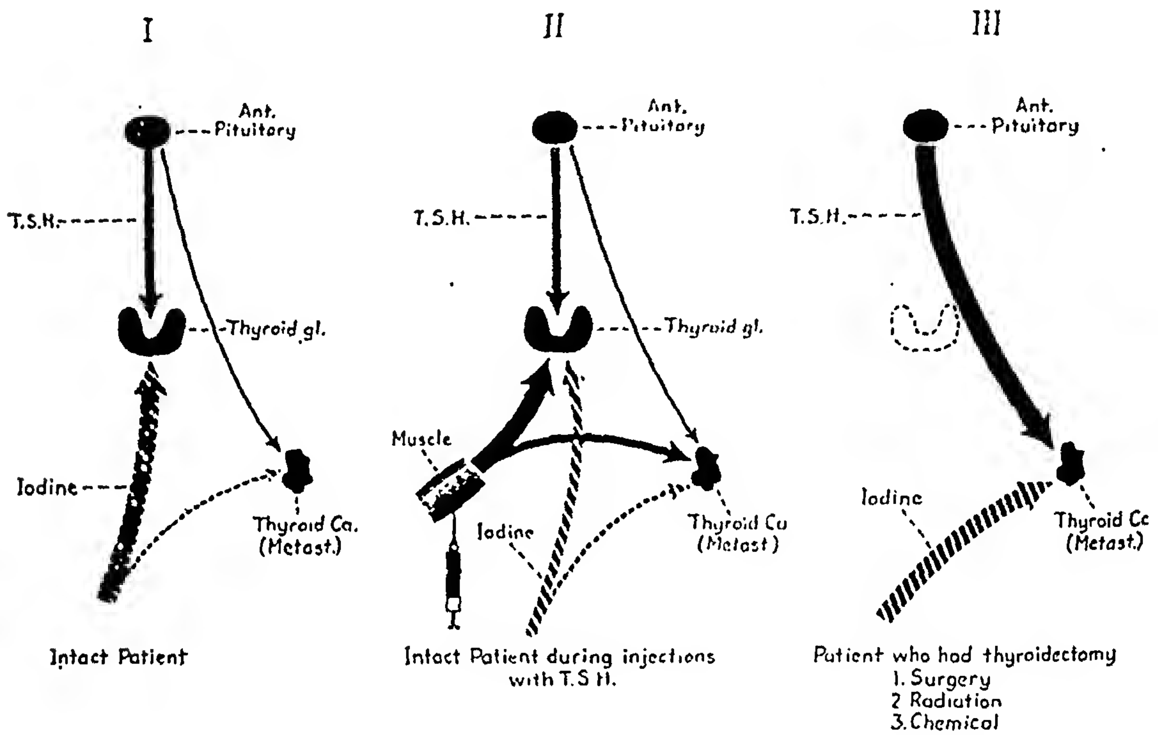

FIG. 8. Diagram representing mechanism of increasing uptake of radioactive iodine by thyroid carcinoma.

We, among others, were of the opinion that thyroid carcinoma metastases possessing an adult type of structure are more likely to pick up iodine than those with an embryonic or anaplastic structure. *This is not always true*. The slide of J. F. (Fig. 5) illustrates a tumor with well differentiated follicles containing colloid, which showed no iodine uptake until after radiation thyroidectomy. However, in patient D. Y. we find radioiodine uptake in nondifferentiated, anaplastic areas as well as in differentiated ones. This is illustrated in the slides shown in Figures 9a, 9b, 9c, prepared by Dr. Titus Evans (6) of Columbia University, using his technique of a combined histologic section and radioautograph.

The determination of the other factors which must influence the pickup of iodine by thyroid carcinoma metastases, in addition to the already demonstrated factors of histologic structure and available supply of T.S.H., requires considerable future investigation.

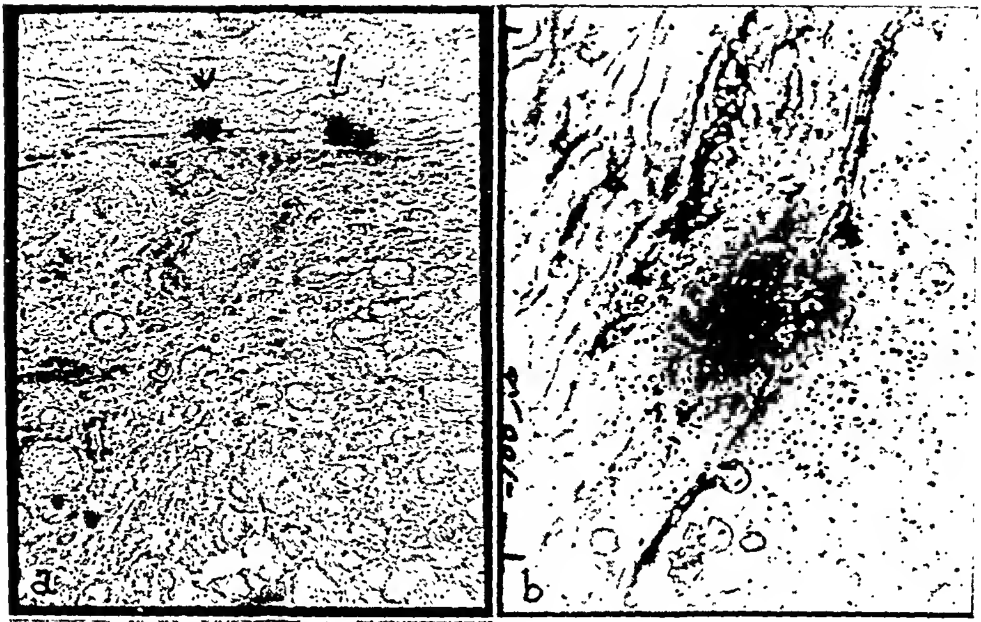

Fig. 9a. Patient D. Y. Section of tumor (× 160) shows well differentiated follicles not showing any uptake of radioiodine: however, there is some uptake of radioiodine in the nondifferentiated tissue.

Fig. 9b. Patient D. Y. Microphotograph (× 640) shows uptake of radioactive iodine in nondifferentiated thyroid tissue.

Fig. 9c. Patient D. Y. Microphotograph of radio-autograph (× 300) showing various degrees of radioiodine uptake by different follicles.

SUMMARY

In 14 unselected cases of metastatic thyroid carcinoma studied with radioactive iodine, uptake of the isotope was demonstrated in 8 (57 per cent). The radioiodine uptake by the metastatic carcinoma was more

closely correlated to the degree of preceding thyroidectomy than to the histologic structure or "type" of the tissue.

In cases in which initial studies with radioiodine do not show concentration in the metastases, such concentration may be induced by either thyroidectomy (surgical, radiation or chemical) or injections of thyrotropic hormone.

ACKNOWLEDGMENTS

The radioactive iodine used in this work was supplied by the Isotopes Division of the United States Atomic Energy Commission, Oak Ridge National Laboratory.

We wish to express our appreciation to Dr. Louis Leiter, Chief of the Medical Division, whose continued encouragement and advice made this work possible. We wish to acknowledge the cooperation of Dr. Daniel Laszlo, Chief of the Neoplastic Service. Technical assistance was given by Gaspar Marov and Barbara Eisner of the Medical Physics Research Laboratory.

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2. Marinelli, L. D.; Foote, F. W.; Hill, R. F., and Hocker, A. F.: Retention of radioactive iodine in thyroid carcinomas, *Am. J. Roentgenol.* 58: 17–30 (July) 1947.
3. Leiter, L.; Seidlin, S. M.; Marinelli, L. D., and Baumann, E. J.: Adenocarcinoma of the thyroid with hyperthyroidism and functional metastases: I. Studies with thiouracil and radioiodine, *J. Clin. Endocrinol.* 6: 247–261 (March) 1946.
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6. Evans, T.: Radioautographs in which the tissue is mounted directly on the photographic plate, *Proc. Soc. Exper. Biol. & Med.* 64: 313–315, 1947.

AGEING IN APPARENTLY NORMAL MEN. I. URINARY TITERS OF KETOSTEROIDS AND OF ALPHA-HYDROXY AND BETA-HYDROXY KETOSTEROIDS[1]

HOWARD B. HAMILTON,[2] M.D. AND
JAMES B. HAMILTON[3] PH.D.

From the Department of Anatomy, Long Island College of Medicine, Brooklyn, New York

THE relation of ageing to the structure and function of the various tissues and organs of the body is in need of objective study. As a move in this direction, an attempt is being made to study in a comprehensive fashion the endocrine status of 57 apparently normal men, with a range in age of 21 to 75 years. It is our further intention to describe the extent to which the endocrine state, as reflected in urinary titers of certain steroid hormones and gonadotropins, is correlated with the degree of development and maintenance of slightly more than a score of special items which are either secondary sex characters or influenced in some lesser fashion by sex hormones.

The present account pertains to the total amounts of ketosteroids excreted by 51 men in this series and to the respective proportions of alpha- and beta-ketosteroids. Brief mention is made, however, of the medical examination and battery of tests used in this survey.

It should be noted at the outset that one of the most important and certainly one of the most difficult aspects of this endeavor has been the selection of a group of normal individuals; and, above all, a *comparable* group of young, middle-aged and old men. The manner in which the problem has been met is of prime consequence and is discussed in the immediately succeeding paragraphs.

Received for publication December 2, 1947.

[1] The term "ketosteroid" is used in preference to the more commonly used designation, "17-ketosteroid." The use of this term is based upon the fact that the compounds recovered by the methods employed include 3-, 17- and 20-ketosteroids (Lieberman and associates (1). The alpha-ketosteroids refer to the hydroxy compounds not precipitated upon the addition of digitonin to the neutral ketonic fraction. The beta-ketosteroids include hydroxy compounds that are precipitated upon treatment with digitonin.

[2] These data were presented before the American Association of Anatomists in 1947 (2).

[3] This work has been supported in part by grants from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council, and from the Nutritional Products Foundation, Inc.

MATERIALS, METHODS AND SUBJECTS

Procurement of Normal Subjects. The original intention was that the old men to be used in this study would be selected from a home for retired sailors, but this institution, like other homes for the aged, was found to house many ailing men who had been admitted because of chronic illness and inability to receive adequate care at home. There seemed to be no satisfactory way to delineate in this population a group which would be a true sample of ageing males and not one loaded with those chronically ill, for example, with men partially disabled by cardiac decompensation or arthritis.

An alternative which had seemed *a priori* to offer good possibilities was the choice of subjects from men employed in an industrial concern or department store. Preliminary inspection of the personnel in two large companies emphasized, however, the extent to which such subjects varied in their living conditions at home and in some instances even in their type of physical work. Because of provisions for retirement of men in the seventh decade of life few persons were available in the older groups.

A third source, a prison, appeared to provide the most satisfactory group of subjects. They lived under an identical regimen. They had not been institutionalized because of physical disability, but because at an earlier time they had disobeyed the law. Furthermore these men were free to devote the required seven to sixteen days apiece to this study. It is superfluous to a humorous degree to emphasize that the routine of life was regulated in a similar fashion for all the men under study. Moreover, the use of such a group avoids the errors inherent in comparisons of age groups selected from different communities, such as would occur in contrasts of young men who are medical students with old men who live in a home for the aged. To be sure, the prisoners are obviously not a cross-section of the population of an area, but the men in different age groups were sufficiently similar as a group to permit comparisons. The prison in which these studies were carried out is a large one in the city of New York. Its inmates were committed for sentences usually of less than three years for crimes such as embezzling, petty larceny, and picking pockets.

Precautions were taken to avoid weighting of the group of subjects with certain types of men who are present in large numbers in prisons. Omitted from this study were homosexuals, narcotic addicts, and those confined to the hospital of the prison or with known histories of tuberculosis or untreated lues. In an attempt to insure further uniformity the survey was restricted to white men.

After observing the above precautions the men were chosen at random simply by drawing names from the list of prisoners in each age group. This was done to avoid selection or rejection on the basis of appearance or

past experience with the men. These men were then informed of the purpose of the study and allowed to accept or reject the invitation to serve as subjects. More than 95 per cent of the men chose to participate, partly from boredom perhaps and partly because of a desire to obtain a thorough medical examination.

The men were quartered in a separate section of the prison while under study, and with one exception cooperated wholeheartedly in the investigation. During the period when they were under study they did not engage in physical activities or in the tasks ordinarily carried out as part of the routine of the prison.

The range in age of the subjects was 21 to 75 years. Eleven men were in the third decade of life, 9 in the fourth, 11 in the fifth, 10 in the sixth, 7 in the seventh, and 3 in the eighth.

Examination of Subjects. Thorough physical examinations of each person were made separately by two of the group of investigators. In some instances the medical history obtained from each subject was supplemented from the files of the prison and hospital. Laboratory work consisted of hematological studies, including total and differential counts of blood corpuscles, hematocrit, corpuscular constants, hemoglobin level and sedimentation rate; chemical studies of the blood; routine urinalysis; an extensive roentgenographic investigation; a limited series of anthropological measurements, including specific gravity of the body and standardized photographic records; and a battery of common and uncommon measurements of the morphological and physiological status of each subject, especially of tests pertaining to the degree of development of various secondary sexual characters and of almost all items known or believed to be influenced by sex hormones. In selected subjects, special surveys were made of the semen and the cardiovascular status; the condition of the prostate as judged by rectal palpation; cytological study of prostatic smears; and histological examination of testicular biopsies. The following descriptions are restricted to procedures used in obtaining data related to the titers of ketosteroids in the urine.

Collection of Urine. Urine for the study of ketosteroids was collected for three to six days. The exact number of days of collection for each man is stated in Table 1. All samples of urine were obtained over consecutive periods of hours and days. As other means of disposal were not provided, no urine was lost inadvertently. Small individual containers were kept in the cell of each prisoner where they were available at all times. At intervals of six hours these bottles were emptied into five-gallon carboys containing 30 per cent sulfuric acid in amounts sufficient to maintain the urine at a pH of 4 or less in order to prevent ammoniacal decay. At the end of each period of collection the amount of acid was adjusted until it

TABLE 1. DATA FOR EACH MAN PERTAINING TO HIS AGE, THE INTERVAL DURING WHICH URINE WAS COLLECTED, AND THE VALUES FOR TOTAL KETOSTEROIDS AND FOR ALPHA- AND BETA-KETOSTEROIDS.

Case number	Age in years	Consecutive hours of urine collection	Ketosteroids (mg./24 hours) *Total (before fractionation of crude neutral fraction)	Total (sum of alpha- and beta-ketosteroids)	Alpha	Beta	Alpha-ketosteroids as % of total sum of ketosteroids	Beta-ketosteroids as % of total sum of ketosteroids	Ratio of beta- to alpha-ketosteroids
1	21	84	14.0	16.0	15.1	0.9	94.5	5.5	5.9
2	22	84	13.7	14.1	12.7	1.4	90.0	10.0	11.0
3	23	144	10.4	11.7	9.1	2.6	77.7	22.3	28.6
4	23	144	6.8	7.0	6.9	0.1	98.8	1.2	1.5
5	23	84	15.5	15.5	14.5	1.0	93.5	6.5	6.9
6	23	120	7.2	7.2	—	—	—	—	—
7	25	84	11.5	11.9	11.6	0.3	97.5	2.5	2.6
8	27	120	10.5	11.5	11.0	0.5	95.6	4.4	4.5
9	27	72	6.1	6.5	5.7	0.8	87.7	12.3	14.3
10	28	120	8.4	8.6	6.0	2.6	69.7	30.3	43.3
11	29	144	8.3	9.2	8.8	0.4	95.7	4.3	4.5
12	30	120	8.2	8.6	6.7	1.9	79.0	21.0	23.4
13	32	120	10.1	10.1	—	—	—	—	—
14	33	144	6.9	7.0	5.9	1.1	84.3	15.7	18.7
15	33	84	15.0	14.8	14.1	0.7	95.3	4.7	5.0
16	33	84	20.5	20.0	18.1	1.9	90.6	9.4	10.5
17	34	84	9.5	10.2	9.2	1.0	90.1	9.9	19.9
18	35	144	5.6	5.2	4.2	1.0	80.7	19.3	23.8
19	38	120	10.6	11.7	9.0	2.7	76.9	23.1	30.0
20	39	144	9.1	9.4	8.9	0.5	94.7	5.3	5.6
21	40	120	6.2	6.0	4.6	1.4	76.7	23.3	32.0
22	41	120	7.8	7.8	6.8	1.0	87.2	12.8	14.7
23	44	84	14.4	14.1	12.7	1.4	90.0	10.0	11.0
24	45	72	4.7	4.9	4.9	<0.1	100.0	0.0	0.0
25	46	144	5.5	5.2	5.1	0.1	98.1	1.9	2.0
26	46	120	11.0	11.0	7.9	3.1	72.1	27.9	39.2
27	46	120	8.1	8.1	—	—	—	—	—
28	47	84	5.9	8.4	8.0	0.4	95.2	4.8	5.0
29	48	120	4.9	5.9	5.2	0.7	87.7	12.3	14.0
30	49	84	8.0	8.9	8.3	0.6	93.2	6.8	7.2
31	49	48	4.5	4.8	4.7	0.1	97.9	2.1	2.2
32	51	144	4.3	5.2	5.1	0.1	98.3	1.7	2.0
33	52	84	10.5	10.7	10.1	0.6	94.5	5.5	5.9
34	53	120	7.8	8.1	7.5	0.6	92.7	7.3	8.0
35	54	120	5.1	6.1	5.7	0.4	94.6	5.4	5.5
36	54	84	8.7	8.9	7.6	1.3	86.0	14.0	16.7
37	55	120	3.9	3.9	3.9	<0.1	100.0	0.0	0.0
38	55	120	4.0	4.4	3.9	0.5	88.6	11.4	13.9
39	57	48	2.2	3.6	2.7	0.9	74.5	25.5	34.2
40	58	120	4.0	4.5	4.1	0.4	92.3	7.7	8.2
41	59	120	4.4	4.8	4.7	0.1	98.0	2.0	2.1
42	60	120	6.6	6.3	6.1	0.2	96.3	3.7	3.9
43	60	120	4.1	3.8	3.5	0.3	91.6	8.4	8.6
44	60	120	3.6	3.8	3.7	0.1	97.5	2.5	2.7
45	62	120	3.1	3.9	3.3	0.6	84.6	15.4	18.4
46	63	48	3.0	4.0	3.6	0.4	89.8	10.2	11.5
47	65	84	5.9	6.9	6.8	0.1	98.5	1.5	1.5
48	68	48	4.6	4.6	4.6	<0.1	100.0	0.0	0.0
49	70	48	2.2	2.1	2.1	<0.1	100.0	0.0	0.0
50	72	144	3.9	4.0	4.0	<0.1	100.0	0.0	0.0
51	75	144	2.4	2.7	2.3	0.4	85.0	15.0	17.6

* The values for total ketosteroids found in the fourth column are those obtained by colorimetric determination of the urinary extract before fractionation, while those in the fifth column represent the sum of the values obtained from direct measurement of the alpha- and beta-ketosteroids.

represented 10 per cent by volume of the total quantity of urine. The urine was extracted within three weeks after collection; collections were made between July and September of 1947.

In the instances listed in Table 2 a second sample of urine was obtained after an interval of several weeks. This was done to check the validity of

TABLE 2. SIMILARITY OF VALUES FOR ORIGINAL AND DUPLICATE COLLECTIONS OF URINE FROM A GROUP OF 12 CASES CHOSEN AT RANDOM.

Case number	Age in years	Number of hours of urine collection	Ketosteroids (mg./24 hours)		Difference (in mg.) between original and duplicate samples
			second sample*	original sample	
3	23	48	11.2	11.7	+0.5
8	27	48	8.8	11.5	−2.7
18	35	72	5.2	5.2	0.0
21	40	48	4.8	6.0	−1.2
38	55	72	5.6	4.4	+1.8
40	58	72	6.4	4.5	+1.9
42	60	72	6.0	6.3	−0.3
43	60	48	4.5	3.8	+0.7
44	60	72	3.8	3.8	0.0
45	62	72	3.7	3.9	−0.2
50	72	48	4.2	4.0	+0.2
51	75	72	2.4	2.7	−0.3

* The interval between the collection of the first and second samples of urine varied from 2 to 8 weeks.

determinations. After it became apparent that the values for many of the prisoners were lower than those for some of the personnel of the medical laboratories and hospital, additional studies were made at intervals in laboratory personnel, thus making certain that the method of extraction and assay continued to give such high values. The ages of the persons not in prison and data pertaining to their titers of urinary steroids are given in Table 6.

Extraction, Separation and Measurement of Ketosteroids in the Urine. Extraction and separation of the ketosteroids followed closely the procedure utilized by Dobriner and associates (3) and colorimetric measurement was done by the method of Zimmermann (4) as modified by Holtorff and Koch (5).

In the calculations the term "total amount of ketosteroids" refers to the sum of the values for alpha- and beta-ketosteroids and not to data obtained by colorimetric examination of the neutral ketonic fraction. Colorimetric measurements of the neutral ketonic fraction were also made, and as can be seen from their tabulation in Table 1 they differ slightly in

TABLE 3. THE AVERAGE DAILY EXCRETION OF TOTAL KETOSTEROIDS SHOWS A PROGRESSIVE DECREASE WITH EACH DECADE OF ADULT LIFE.

Age in years		Number of men in group	Total ketosteroids (mg./24 hours)	
Range	Average*		Average	Range
20-29	24.6	11	10.8	5.9-15.5
30-39	34.1	9	10.1	5.2-20.0
40-49	43.6	11	7.7	4.8-14.1
50-59	54.8	10	6.0	3.6-10.7
60-69	62.1	7	4.8	3.8- 6.9
70-75	72.3	3	2.9	2.1- 4.0

* The averages per decade are computed from individual values listed in column 5 of Table 1.

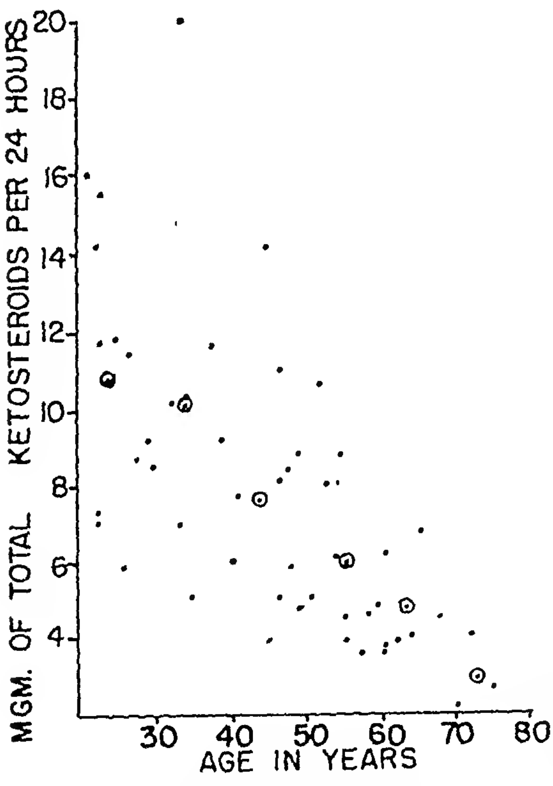

FIG. 1. Progressive decrease with age in the excretion of urinary ketosteroids. Dots indicate the titer for each man, circles the average for the men in that decade of life.

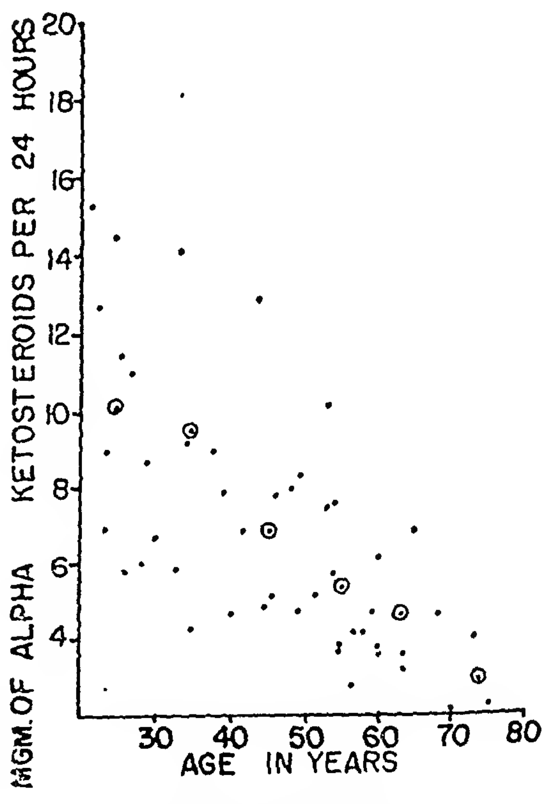

FIG. 2. Showing a decrease with age in average daily excretion of alpha-ketosteroids almost parallel with the decrease with age in the total excretion of ketosteroids, and accounting for much of this reduction.

many instances from the sum of the values for the alpha and beta fractions that were obtained after further purification.

It is to be noted that the values for alpha- and beta-ketosteroids are obtained by direct measurements of each fraction, whereas others (6, 7) have measured one fraction and calculated the other by subtraction from the value for the ketonic fraction. The beta fraction refers to hydroxy substances precipitated upon addition of digitonin to the neutral ketonic fraction, and the alpha fraction consists of those hydroxy compounds that do not precipitate when treated with digitonin.

RESULTS

Accuracy of Methods. A check on the possibility of error in the collection and preservation of urine and in the accuracy of the measurements of the

TABLE 4. PROGRESSIVE DECREASE OF THE AVERAGE EXCRETION OF ALPHA- AND BETA-KETOSTEROIDS IN THE SUCCESSIVE DECADES OF LIFE (COLUMNS 4 AND 6) AND THE RATIOS OF THE BETA- TO TOTAL, AND BETA- TO ALPHA-KETOSTEROIDS (COLUMNS 9 AND 10).

Age in years		Number of men in group	Ketosteroids (mg./24 hours)				Ratio of		
			Alpha-		Beta-		Alpha- to total keto-steroid	Beta- to total keto-steroid	Beta- to alpha-keto-steroid
Range	Average		Average	Range	Average	Range			
20–29	24.8	10	10.1	5.7–15.1	1.1	0.1–2.6	91.0	9.0	12.3
30–39	34.3	8	9.5	4.2–18.1	1.3	0.7–2.7	86.3	13.7	16.5
40–49	45.5	10	6.8	4.7–12.7	0.9	0.1–3.1	90.2	9.8	12.7
50–59	54.8	10	5.5	2.7–10.1	0.5	0.1–1.3	92.0	8.2	9.7
60–69	62.1	7	4.6	3.3– 6.1	0.2	0.1–0.3	94.0	6.0	6.7
70–75	72.3	3	2.8	2.1– 4.0	0.1	0.1–0.4	95.0	5.0	5.9

ketosteroids was provided by assays on duplicate collections of urine from a few men chosen at random, except that most of them were in the later years of life where study seemed needed most. Agreement between the values for the first collection of urine and those of the second determination was fairly close as shown in Table 2. The greatest differences in any of the cases are less than the expected variation from day to day in normal men, which may amount to as much as 5 milligrams (8, 9, 10, 11).

General Aspects of Data. The total amount and the quantities of alpha- and beta-ketosteroids excreted per twenty-four hours are listed for each patient in Table 1. Tables 3 and 4 present the average and range values for each decade of life. Figures 1, 2, and 3 show the values for each individual in black dots and the average excretion for the men in each decade of life by dots within circles.

From these tables and graphs it can be seen that in all three categories of steroids, that is, in the alpha- and beta-ketosteroids and in the sum of these fractions, the average titers declined with age in a progressive and pronounced fashion. The excretion of total and alpha-ketosteroids diminishes with each decade. The average values for the beta-ketosteroids decrease similarly except for a peak in the fourth decade. This exception appears to be a fortuitous one to be expected when small segments of the

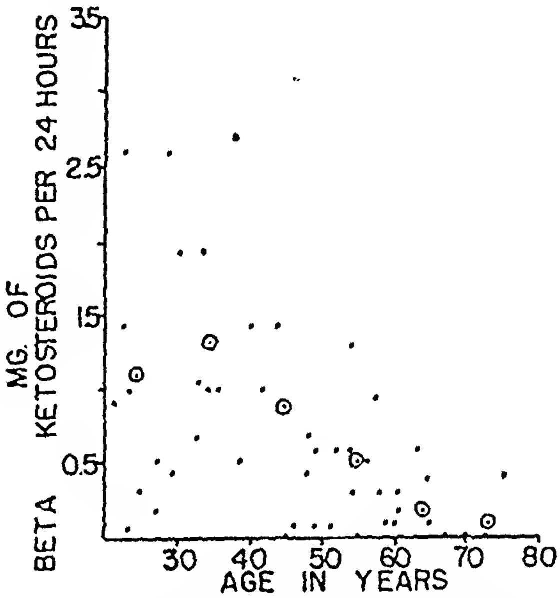

FIG. 3. The excretion of beta-ketosteroids also decreases with age.

data are considered; it is related to the absence in this decade of very low values, several of which are found in the preceding and in all succeeding decades.

Values for Sum of Alpha- and Beta-ketosteroids. The decline in values for the average daily excretion of total ketosteroids follows almost a straight line decade by decade. In comparison with the output in the third decade, that in the seventh decade is less than one-half and that in the eighth decade only 27 per cent (Table 3).

Values for Alpha-ketosteroids. The fall in total output of ketosteroids is due mostly to a loss in alpha-ketosteroids which comprise on the average more than 90 per cent of the ketosteroids. Therefore, the curve of decline in alpha-ketosteroids (Fig. 2) follows closely that of the total ketosteroids (Fig. 1). In comparison with the average in the third decade, the excretion in the seventh decade was more than halved and that in the eight decade reduced to 28 per cent (Table 4).

Values for Beta-ketosteroids. With the passing years the excretion of

beta-ketosteroids diminishes (Fig. 3) in much the same fashion as that of alpha-ketosteroids and the sum of these two fractions. An exception to this generalization is the average value for the fourth decade which may not be truly representative of this age because of reasons stated before. In terms of percentage decrease from the average value in the third decade, that in the sixth decade was less than half, that in the seventh less than one-fifth, and that in the eighth decade only 9 per cent.

Variability from individual to individual was the rule as regards the proportion of beta compounds in the total ketosteroid ouptut (Fig. 4). It was

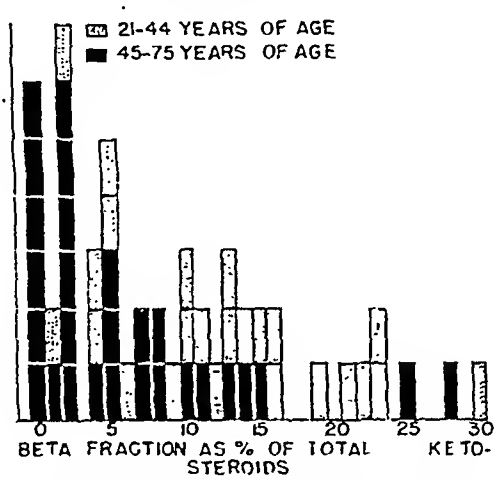

FIG. 4. Graphic portrayal of the effect of ageing upon the percentage of ketosteroids excreted as beta compounds. Each block represents the total for one man.

less than 5 per cent of the total in 31 per cent of the entire group of men; from 5 to 10 per cent in 34 per cent of the men; and above 10 per cent in the remaining 35 per cent.

The percentage of the total ketosteroids formed by the beta fraction appears to be related to age when the data are considered in averages by decades (Table 4), or in two larger groups of men, 21 to 44 and 45 to 75 years of age. Less than 5 per cent of the ketosteroids were excreted as beta compounds in 19 per cent of the men who were 21 to 44 years old but in 44 per cent of those 45 to 75 years old. Of the 13 men in the entire series whose beta fractions were so low as to amount to no more than 2 per cent of their total ketosteroids, 11 were 45 or more years of age. Expressed as percentages, 41 per cent of the men over 45 years of age had only 2 per

cent or less of beta compounds whereas only 11 per cent of those 21 to 44 years old had such a small proportion of beta compounds (Fig. 4).

Conversely a beta fraction in excess of 15 per cent of the total output was observed in 42 per cent of those less than 45 years of age, in contrast to only 7 per cent of those 45 years of age or more.

In previous investigations (12, 13, 14) the proportion of beta-ketosteroids in the total output has not exceeded 15 per cent except in an occa-

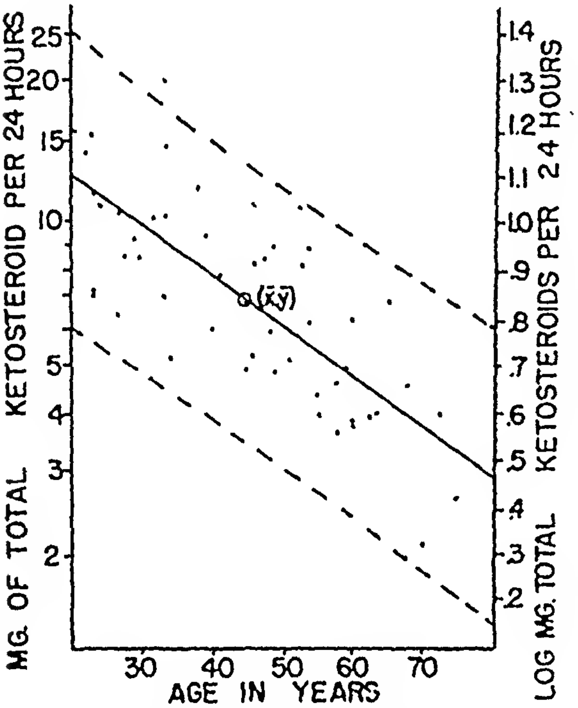

FIG. 5. Graphic representation of the values for total ketosteroid titers statistically analyzed and summarized in Table 5. The x coordinate has been plotted arithmetically, and the y coordinate logarithmically.*

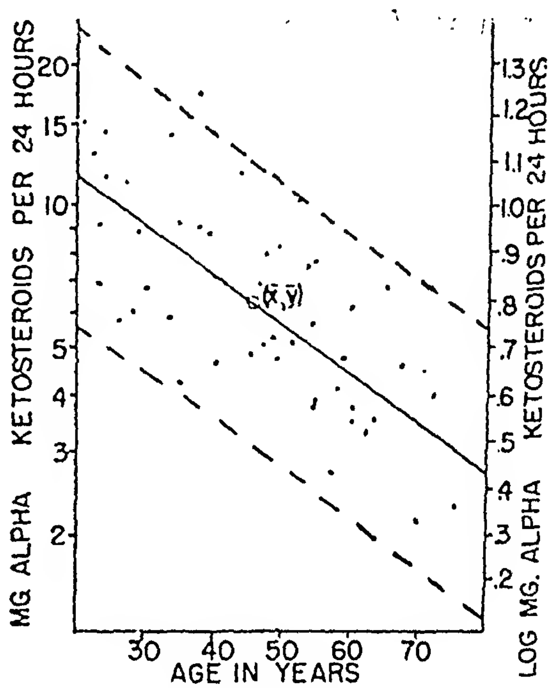

FIG. 6. Graphicre presentation of the statistical analysis of the values for alpha-ketosteroids.

* Conversion of ketosteroid values to logarithmic equivalents facilitates comparison of the curves for total, alpha- and beta-ketosteroid output or for titers obtained in individual men; comparisons can be made with other diverse measurements such as weight, height, and specific gravity of the subject, since the logarithmic equivalents are independent of units of mensuration; adjustment is made for the variation in output as a function of age, so that 2 instead of 3 variables remain for analysis. The line of regression was found in the usual manner by substituting values of x in the equation, solving for y, plotting these values, and drawing a curve through the points located. The points determining the zone of error are found by adding to and subtracting from various y values twice the standard error of estimate for those y values, plotting these points with respect to the corresponding x value, and drawing a curve through the points (broken lines on the graph). In a group of cases selected at random where distribution is normal, 95 per cent of such cases will fall within the zone of error. $(\bar{x}, \bar{y})$ on the graph represents the average age and ketosteroid titer for the entire group, i.e., the mean.

sional case (15), whereas eight such instances were found in the present series.

Statistical Analysis of the Entire Series. Since the total number of subjects in the study is relatively small it seemed desirable to analyze the data as a whole in addition to discussing the results by decades. The advantage in the former method is that errors in sampling are less likely to appear, but the latter method has its value here where the groupings are

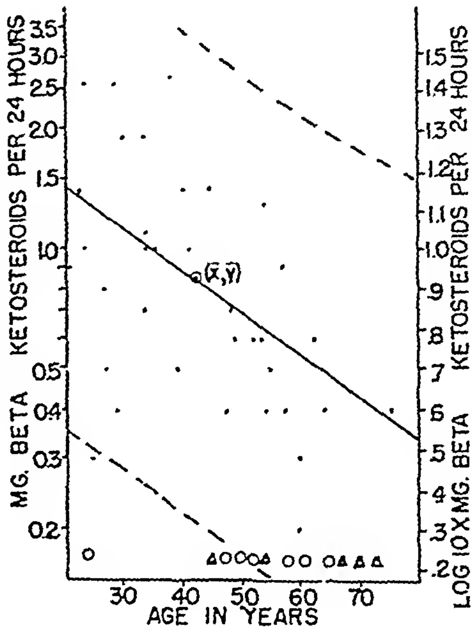

FIG. 7. Graphic portrayal of the statistical analysis of the beta-ketosteroids. A factor of 10 has been employed in the logarithmic scale for convenience in plotting the graph. The open circles and triangles at the bottom of the graph represent values that were left out of the calculations. The circles are for values of 0.1 mg. and the triangles for those values less than 0.1 mg.

so small that strict statistical analysis cannot be made. It should be noted that the chief difference in the data treated as a whole from that given simply as average figures by decades, is that in the latter method the very lowest values for the beta-ketosteroids are omitted since they seem to form a discontinuous series with respect to the remaining data for this fraction. Further, as will be seen later, when the very low figures are omitted from the calculations, the ratios of beta- to total and beta- to alpha-ketosteroids do not fall with increasing age as they do when analyzed by averages for each decade. This apparent discrepancy is explained, of course, by the fact that the figures in the later decades are weighted by the low titers obtained in several men. Figures 5, 6, and 7 show graphically the curves obtained by such statistical treatment of the data for the excretion of total, alpha- and beta-ketosteroids. Table 5 lists the values of the means, slopes, and intercepts for the equation of these curves as well as the standard errors, standard deviations, and standard

errors of estimate for the average total, alpha-, and beta-ketosteroid output.[4]

In comparing the three equations derived, it can be seen that the slopes are all very nearly equal which indicates that total, alpha-, and beta-ketosteroid outputs decline at similar rates as age increases, and that the ratios of alpha- to total, beta- to total, and beta- to alpha-ketosteroids are equal throughout life.

The significance of the apparent discontinuity of values discussed before is not clear. Whether it is due to errors in collection, extraction, or assay of the steroids cannot be determined, although these possibilities do not seem likely. A probable explanation is that there is a small group of men, increasing in number with age, who excrete very low amounts of beta-ketosteroids. Confirmation of this finding and its possible relationship to function of the adrenal cortex await further investigation. Whatever the cause, the conclusion that the average decrease with age is more rapid in beta- than in alpha-ketosteroids should await substantiation in studies of additional patients in such numbers as to exclude the possibility of error that is inherent in the use of small samplings.

Ratio of Alpha- and Beta-ketosteroids. The proportion of the total ke-

[4] The equations (of the type $y = a + bx$) are derived by the method of mean squares using the following expressions: Sx, Sx^2, $(Sx)^2$, Sy, Sy^2, $(Sy)^2$, Sxy, and $SxSy$, where S is summation and x and y are values for age and ketosteroid output respectively. The means, $\bar{x}$ and $\bar{y}$, equal Sx/n and Sy/n; a, the y intercept, equals $y - b\bar{x}$; b, the slope, equals

$$\frac{Sxy - SxSy/n}{Sx^2 - (Sx)^2/n}.$$

The standard error for $\bar{y}$ is

$$\frac{\text{Standard Deviation}}{\sqrt{n}}.$$

Where $[y^2] = Sy^2 - (Sy)^2/n$, $[xy] = Sxy - SxSy/n$ and $[x^2] = Sx^2 - (Sx)^2/n$, other quantities are derived as follows: the standard deviation for $\bar{y}$ is

$$\sqrt{\frac{[y^2]}{(n-1)n}}$$

and the standard error of estimate is

$$\sqrt{\frac{[y^2] - [xy]^2/[x^2]}{n-2}}.$$

tosteroid output represented separately by the alpha and beta fractions, and the relative proportions of these two fractions one to the other, are tabulated in Table 4 for data arranged by decades.

The average decrease with age in values for beta-ketosteroids is more pronounced than the reduction in total ketosteroids even though the decrease in the total ketosteroids by the eighth decade amounted to about three-fourths of the output in the third decade. The average excretion of beta-ketosteroids, which constituted about 10 per cent of the total quantity of ketosteroids in the third decade, was only 5 to 6 per cent in the

TABLE 5. SUMMATION OF THE VALUES OBTAINED IN THE STATISTICAL ANALYSIS OF THE EXPERIMENTAL DATA OF TABLE 1.

Keto-steroid	$\bar{x}$ Average age (years)	$\bar{y}$ Average titer in mg./24 hours	a y intercept	b slope	Standard deviation of $\bar{y}$	Standard error of estimate of $\bar{y}$
Total	44.75	6.98 ±0.15	2.32	−0.0106**	1.07	0.15
Alpha	45.44	6.28 ±0.16	2.27	−0.0104**	1.08	0.15
Beta*	42.17	0.93 ±0.06	1.38	−0.0106**	0.35	0.30

The equations for the lines of the graphs in Figures 5, 6, and 7 are of the general form for linear regression: $y = a + bx$, where x and y are the linear coordinates (in this case representing age and log mg. of ketosteroid output per 24 hours respectively), a is the y intercept, and b is the slope of the curve, in this case negative. In the table, $\bar{x}$ represents the average age for the entire group and $\bar{y}$ the average daily ketosteroid output.

* 12 values omitted (cases 4, 24, 25, 31, 32, 37, 41, 44, 47, 48, 49, and 50) for reasons given elsewhere.

** Significantly different from zero at the 1 per cent level (Snedecor, *Statistical Methods*, 1946, Chapter 6 and Table 10.7).

seventh and eighth decades. Because of the greater average decline in beta- than in alpha-ketosteroids the ratio of the beta to the alpha compounds seems to decrease slightly in the later years (Table 4).

Relationship between Ketosteroid Values and the Mass of Body Protoplasm. An analysis still in progress shows a high degree of correlation between the quantities of ketosteroids in the urine and the weights of the individual men. In every decade the output of ketosteroids was less, the lighter the men. Among the men under study, who had as a group very little tendency to obesity, the decline in values with age was definitely associated with a decrease in body weight. More detailed consideration of the relationship between titers of urinary ketosteroids and body weight is reserved for future consideration which will also include height, specific gravity, surface area and other pertinent features.

Individual Variability in Values of Ketosteroids. There is great variance in values from individual to individual, as has been reported previously (8, 10, 11, 12, 14–26).

DISCUSSION

Several points are worth noting in comparing the results in the present survey with observations made by other workers. The values for the men in this series are slightly lower than some of those reported by other investigators, most of whom have dealt with more restricted age groups, particularly with young men in the third and fourth decades of life. This difference between our results and those of others does not appear to be due to a loss of ketosteroids in the present study, since the results obtained with our methods on the urine of normal young adult males living outside the prison yielded values comparable with those of other workers. These data, obtained on the personnel of the medical laboratory and hospital, are shown in Table 6.

TABLE 6. VALUES FOUND IN NORMAL PERSONNEL OF THE LABORATORY AND HOSPITAL AS REGARDS THE AVERAGE DAILY EXCRETION OF URINARY KETOSTEROIDS.

Case	Age in years	Number of hours of urine collection	Ketosteroids (mg./24 hours)
A	18	72	15.1
B	18	96	17.1
C	24	96	10.0
D	25	120	10.5
E	26	96	18.0
F	27	96	17.2
G	30	72	21.0
H	47	48	10.4

One explanation for the difference between the data of prisoners and those of other subjects may be the fact that both the food and routine of life in the prison were unpalatable to the prisoners. With few exceptions the men disliked the diet and refused portions of each meal. Many complained that they were always hungry, and it was the usual impression of most prisoners that they had lost weight during imprisonment.

It should be noted too that the physical activities of the men were slight during the period of study.

Another matter to be considered is the possibility that the length of stay in prison might be correlated with the titers of ketosteroids. Figure 8 shows that there is no such relationship between these items. Not elimi-

nated is the likelihood that many of these men were not in good health even prior to commitment in prison. Some had been jailed as a result of sequelae of bouts of alcoholism and a few had been hiding from the law. It is possible, then, that a lack of well-being involving a loss of weight in several instances may explain in part the somewhat lower values than those usually reported in young men.

It has been found (17, 18, 19, 27, 28, 29) that in various diseases and instances of poor health the output of urinary ketosteroids may be lowered considerably. The subjects of this study, with a few exceptions, cannot be considered to be in ill health. All were participating actively in routine prison affairs prior to the beginning of the survey. Table 7 shows pertinent clinical findings in those members of the group who might be considered

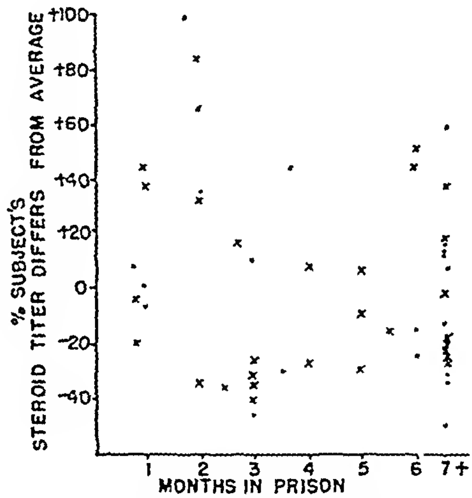

FIG. 8. Showing that there is no correlation between the length of each subject's internment in prison and the percentage by which his titers of total urinary ketosteroids differ from the average for the men of his age. The data for the younger men are represented separately by crosses in order to distinguish them from the values for older men: crosses represent those subjects less than 50 years of age.

to be below par in health. No disease was observed in the majority of the men in the third decade, that is, in the younger men for whom higher values might have been expected; the titers of 2 men with somewhat abnormal clinical findings did not differ much from the average for the decade. Similarly, examination of the right-hand column in Table 7 shows that the values for ketosteroids did not tend to be less than the average for the decade in men of other ages in whom one or another abnormality was detected in the medical examination. This is evidence that the low titers in these groups did not arise from illnesses of certain men.

Another matter to be considered and then disregarded in this connection is the definitely lower values in specimens collected in winter as compared to those obtained in summer (18). All collections for this study were made during summer months.

With regard to claims that a climacteric-like state occurs in men, it

TABLE 7. STATUS OF THE MEN WHO DESERVED THE MOST CONSIDERATION AS TO WHETHER OR NOT THEY WERE POSSIBLY OR ACTUALLY ILL. THE TITERS OF KETOSTEROIDS IN THESE CASES DO NOT DIFFER APPRECIABLY FROM THE AVERAGE FOR THEIR DECADE.

Case	Age in years	Sedimentation rate in mm./hour (Westergren)	Medical Status	Difference between ketosteroid titer and average for age group (mg./day)
2	22	—	Controlled diabetic.	+3.3
6	23	2.8	Extensive rash on flexor surface of arms and forearms, on popliteal spaces, inner surface of thighs, inferior aspect of buttocks and dorsum of neck. Skin of these areas thickened, encrusted and scaling, with crusts raised from skin surface. Where scales flaked off, area was reddened and fluid oozed from the naked surface.	−3.6
12	30	7.0	Extensive acne on back.	−1.6
15	33	2.0	Chronic postnasal drip.	+4.7
16	33	5.0	Scabies; systolic murmur judged to be grade ii; extrasystoles.	+9.9
18	35	5.0	Irregular cardiac rhythm (not fibrillating).	−4.9
26	46	29.0	Faint pink macular rash on trunk with no papular elevation but otherwise suggestive of hives.	+3.3
29	48	12.0	Right leg amputated as a result of trolley accident. Many bones broken at this time.	−1.8
30	49	27.0	Scoliosis; dribbling of urine (collection	+1.2
33	52	2.5	bag). Right hydrocele tapped repeatedly about 10 years ago.	+4.7
35	54	13.0	Pain in shoulder joints on movement of arms above horizontal position; pain to deep palpation over deltoid areas; no abnormalities visible by x-ray.	+0.1
39	57	—	Soft penile chancre; treated for lues 28 years ago for a period of 16 months.	−2.4
41	59	54.4	Varicose ulcers on right lower leg.	−1.2
42	60	27.0	Mild cardiac decompensation.	+1.5
43	60	24.0	Healed varicose ulcers of legs.	−1.0
44	60	31.0	Left leg amputated following extensive varicose ulceration; varicose ulcers on right lower leg.	−1.0

TABLE 7 (Continued)

Case	Age in years	Sedimentation rate in mm./hour (Westergren)	Medical Status	Difference between ketosteroid titer and average for age group (mg./day)
45	62	35.0	Chronic hypertrophic arthritis for many years.	−0.9
46	63	—	Extreme fungus infection of left foot; foot swollen to twice normal size.	−0.8
47	65	28.5	Diarrhea of unknown etiology for few days prior to participation in survey.	+2.1
48	68	—	Questionable cardiac enlargement; blood pressure 190/80; ?aortic valvular disease.	−0.2
49	70	—	Marked systolic murmur heard over entire precordium and all normal heart sounds absent; no decompensation evident; blood pressure 150/90.	−0.8
50	72	11.0	Auricular fibrillation; receiving digitalis; no decompensation evident; blood pressure 160/80.	+1.1
51	75	41.0	Operation some years ago for varicose ulcers of leg: blood pressure 160/80.	−0.2

should be noted that the values for ketosteroids in the present series of men indicate that a gradual rather than a sudden decline of output occurs with ageing. Until information is obtained as to the excretion of gonadotropins and androgens by these subjects the data at hand are insufficient to allow definitive statements.

The literature seems to contain no survey of the excretion of ketosteroids in the later decades of life or of the effect of ageing upon those values. Venning and Kazmin (10) in a study of 14 males ranging in age from 20 to 51 years reported the average excretion of 16.6 mg. of ketosteroids per twenty-four hours, with a range of 10 to 25 mg.; two other men aged 77 and 81 excreted 10.0 and 10.6 mg. per twenty-four hours. These figures do not appear to be significantly less than their findings in the younger men. Similarly, Luft's (21) series, which contains 15 subjects under 40 and 8 subjects over 40 years of age, shows no significant falling off of values with increased age. The lowest titers were found in the 2 youngest and 2 oldest subjects; the values for these 4 persons aged 16, 22, 71, and 82 years were 15.5, 19.8, 14.3, and 18.6 mg. respectively, considerably less

than the average of 26.2 mg. for the entire group. In Salter's (15) series of normal males, only two are past the fourth decade; and the values for these, aged 42 to 58 years, are 23 and 12 mg., compared to an over-all average of 15.3 mg. for 15 subjects. As each of these studies contains comparatively few subjects in the decades beyond the fourth, it is doubtful whether one is justified in drawing conclusions from them. In the investigation carried out by Chou and Wang (17) there is a decline in ketosteroid values with increasing age, but here again there are only 4 men in the fifth, 2 in the sixth, and 2 in the seventh decades. Wooster (25) investigated the titers of ketosteroids in a group of 49 subjects whose range of age was the third to sixth decades. The mean values for one decade compared to another were not significantly different in his series, although he noted that in terms of milligrams the variation in output from individual to individual tended to decrease with age.

Data in the literature that bear upon the relation of age to alpha- and beta-ketosteroids are even more limited than those pertaining to the total output. Most studies of alpha- and beta-ketosteroids have been made with reference to their excretion in disease (6, 23, 30, 31) particularly in hyperplasia or neoplasia of the adrenal cortex, whereas the present study is concerned with the amounts of each fraction excreted by normal individuals and the relations of such values to the endocrine state in later life. The findings in the present study would appear to be at variance with the data of Salter (15), in that with ageing a) the change in the excretion of alpha compounds more or less parallels the decline in values of total ketosteroids (Tables 3 and 4; Figs. 1 and 2); and b) the average beta values also tend to decline in amount with increasing years (Tables 3 and 4; Figs. 1 and 3). As there are only 2 subjects over 40 years of age in Salter's series, valid comparisons cannot be made with his data.

Finally, it should be noted that as the titers of total ketosteroids decrease with age there is no great relative increase in the amount of either alpha or beta compounds that would suggest compensatory function by the adrenal cortices in response to any loss of testicular function.

SUMMARY

The effect of ageing upon the excretion of urinary ketosteroids and upon the respective amounts of the alpha- and beta-ketosteroids has been studied in a group of 51 apparently normal men, 21 to 75 years of age, all of whom were living under similar conditions. In successive decades of life the average daily excretion of ketosteroids decreased progressively. The material extent of this reduction in output is shown by the fact that in comparison with the quantity present in the third decade, that present in the seventh decade was less than half and that in the eighth, only 27 per cent.

The major part of this decline is due to a loss of alpha-ketosteroids which accounts in general for over 90 per cent of the total quantity of urinary ketosteroids. The curve of decrease in alpha-ketosteroids parallels closely that for the total output of ketosteroids.

The excretion of the beta-ketosteroids also decreased with age. Very low values were uncommon in the early years of life but frequent in men in the sixth and seventh decades.

In this series of men, who had very little tendency to obesity as a group, the output of ketosteroids in all decades of life was correlated with the body weight of the individual man.

ACKNOWLEDGMENT

The authors are indebted to Dr. Peter Amorosa, late Commissioner of Crime in New York City; Dr. Herman Baxt, resident physician; Warden Johnson, and the men who were subjects in this study at Riker's Island Penitentiary. We wish also to acknowledge gratefully the aid of three medical students, Jacob Katz, Irwin Katzka and Gerald Rodman; the advice of Dr. Konrad Dobriner as to the procedures used in the preservation of urine and the extraction and assay of ketosteroids; and the help in statistical treatment of the data by Drs. Robert Lehman and John Tukey.

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EXPERIMENTAL ALTERATION OF THE HUMAN OVARIAN CYCLE BY ESTROGEN*

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THERE are relatively few studies of the effect of estrogen on the human ovarian cycle. Sturgis and Albright (1) and Sturgis and Meigs (2) demonstrated that ovulation was inhibited in women when estrogen was administered in the early part of the menstrual cycle. This inhibition evidently depended upon early administration of the estrogen (before the sixth day) as well as upon the dosage employed. Their primary interest was in the relief of dysmenorrhea which was obtained by eliminating the luteal phase from the cycle.

They found that bleeding usually occurred from twelve to fourteen days following a single 10 mg. dose of estradiol dipropionate. However, if the dose were repeated in ten days the expected bleeding would be delayed and by suitable timing of the estrogen administration, bleeding could be delayed for as long as three months. Thus, by alternating intervals of ten or fourteen days between doses, it was possible to bring about bleeding at predictable intervals of two weeks, four weeks or eight weeks.

Gillman (3) described the effect of single doses of estradiol benzoate on the length of the human menstrual cycle. He found that small doses (.01 to 0.5 mg.) had little effect but when a dose of 5 mg. was given on the eighth day of the cycle there was an inconsistent effect, some cycles being shortened to nineteen days and others lengthened to forty-five days. Gillman did not report any endometrial biopsies to explain how the ovarian cycle had been affected. Fluhmann and Hoffman (4) failed to produce any alterations in the cycle with 0.05 to 0.10 mg. of estrogen daily. Westman (5) claimed that estrogenic therapy favored luteal function but his evidence was equivocal.

The present study was undertaken to determine in what manner the human cycle is altered by the administration of estrogen. Information relative to the ovarian cycle was obtained by weekly endometrial biopsies.

METHODS

Each of the women selected for this study presented a history of a nor-

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mal menstrual rhythm. On most of the subjects[1] it was possible to obtain weekly endometrial biopsies for consecutive months, before, during, and after treatment. Some of the patients were seeking relief from dysmenorrhea. Stilbestrol was the estrogen used and it was given orally according to three general schedules: Early in the cycle during the proliferative phase; late in the cycle during the luteal phase; and continuously during the cycle.

EXPERIMENTAL RESULTS

EARLY TREATMENT

Six patients were given a single 20 mg. dose of stilbestrol early in the menstrual cycle (4th, 5th, or 6th day), as shown in Figure 1. The next menstrual period was delayed, the interval varying from thirty-three days for M. J. to forty-two days for M. W., with an average duration of thirty-eight days for the group. As indicated in the graph, the control menstrual intervals before and after treatment were normal and averaged thirty and twenty-nine days respectively.

Biopsies, taken after the estrogen treatment, revealed secretory endometrial changes in 5 of the 6 patients (M. J. being the exception). Prolongation of the menstrual interval was associated with a delay in the onset of the secretory pattern. The duration of the luteal phase at the end of the cycle was the same as that seen in a normal untreated cycle.

A similar study was made on 5 women who complained of dysmenorrhea (Fig. 2). The menstrual interval was prolonged from thirty-four to thirty-seven days, as compared to a normal twenty-eight to thirty day cycle. In 4 of these patients, the premenstrual biopsy showed secretory endometrium and the dysmenorrhea was not affected by the estrogen. One patient (M. S.) however, did have an anovulatory cycle following the 20 mg. dose of stilbestrol. In only two or three instances was there any nausea following the administration of the drug.

Seven to ten days after the stilbestrol was given, 5 of the patients in these two groups (Figs. 1 and 2) experienced slight vaginal bleeding that lasted from two to four days. Since the bleeding was so scanty and was not associated with changes in the endometrial pattern, it was considered as an estrogen-withdrawal bleeding rather than a menstrual period. In patient M. S. (Fig. 2) it recurred on the twenty-seventh and twenty-ninth days of the cycle. These episodes of slight bleeding (usually not enough to require a pad) may have been similar to those that led Gillman to state that the cycle was shortened in 2 of his cases. Four of Gillman's patients treated with 5.0 mg. doses of estradiol benzoate experienced cycles of thirty-one to

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[1] Through the courtesy and cooperation of Doctor Adolph Soucek of Mount Pleasant State Hospital, patients were made available for these observations.

forty-five days, comparable to the majority of our cases. Sturgis and Meigs also found that a 10 mg. dose of estradiol dipropionate would prolong the interval. Patient No. 3 in their series experienced slight bleeding on the fifteenth day and then had a painful (ovulatory) menstrual period forty-six days after the previous normal menses.

THE EFFECT OF A SINGLE LARGE DOSE OF ESTROGEN GIVEN EARLY IN THE MENSTRUAL CYCLE (DELAY IN OVULATION)

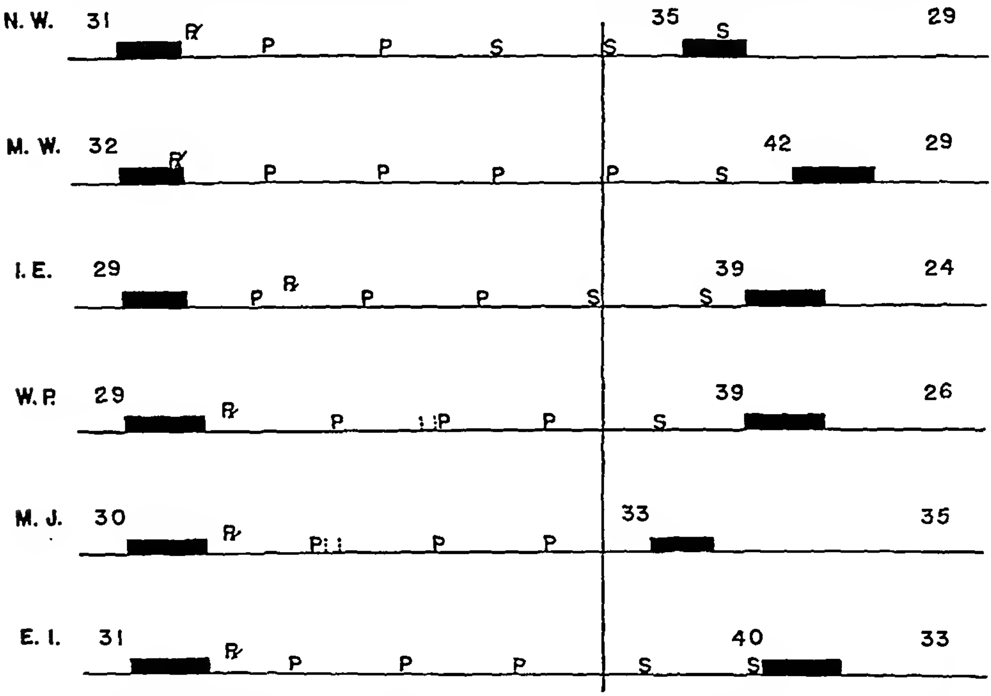

FIG. 1. Graphic representation of the effect of 20 mg. of stilbestrol given orally early in the cycle. The numbers represent the length of the cycle before, during and after treatment. The letters represent the stage of the endometrium, P for proliferative and S for secretory, as revealed by weekly biopsies. The vertical line is 30 days after the onset of the previous period. The dotted lines represent days of slight vaginal bleeding, whereas the menstrual periods are denoted as solid black bars.

Since the present findings are in apparent agreement with those of Sturgis and Meigs and of Gillman it may be stated that a single dose of estrogen given early in the menstrual cycle will usually delay but not inhibit ovulation and will thereby increase the length of the cycle.

PROLONGED TREATMENT

Sturgis and Albright (1) found that when estrogen was given continuously from the fourth or fifth day of the cycle until the eighteenth or twen-

tieth day, ovulation was prevented and the luteal phase of the endometrium did not develop. Under these conditions dysmenorrhea was completely relieved. Either estradiol benzoate 1.6 mg. intramuscularly every third day or oral stilbestrol 1 mg. daily was equally effective. Under these conditions of therapy they found that dysmenorrhea could be relieved (ovulation prevented) on alternate months but not in consecutive cycles. Randall and Odell (6) found that ovulation was inhibited and dysmenor-

THE EFFECT OF A SINGLE LARGE DOSE OF ESTROGEN
GIVEN EARLY IN THE MENSTRUAL CYCLE

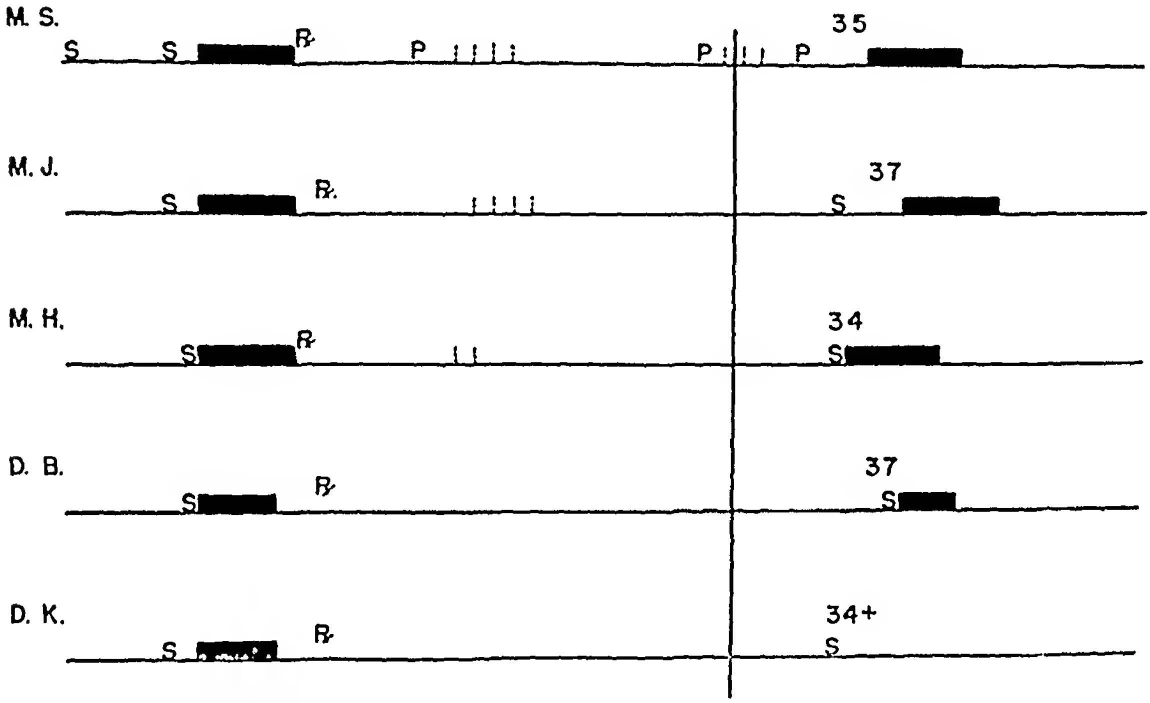

FIG. 2. Graphic representation of the effect of 20 mg. of stilbestrol given early in the cycle as a single oral dose. The vertical line is 28 days after the onset of the previous period. The rest of the graph is the same described for Figure 1. Biopsies were obtained only in the premenstrual period.

rhea relieved when 1 mg. of stilbestrol was given daily for twenty days following cessation of menstruation.

A group of patients was given stilbestrol to determine whether relief from dysmenorrhea could be obtained with an oral estrogen for three successive months. Starting at the end of a menstrual period, 1 mg. of stilbestrol was given daily for ten days, 2 mg. daily for the next ten days and 3 mg. daily for the third ten days. Bleeding usually occurred within three days after the last 3 mg. dose and the dysmenorrhea was completely relieved (Fig. 3). One attempt with hexestrol on a 3, 6 and 9 mg. increasing dose schedule was ineffective, whereas the patient obtained relief the fol-

lowing month with stilbestrol. Endometrial biopsies obtained in the third month of treatment in 3 of these patients revealed a proliferative endometrium, as would be expected from the findings of Sturgis and co-workers. There was no evidence of hyperplasia or more profuse bleeding in these patients followed for three successive months.

The side effects of stilbestrol were apparent in some instances. Two patients noticed a definite weight gain (5 to 11 pounds) and edema during the latter part of the treatment, and experienced a definite diuresis with

ESTROGEN GIVEN CONTINUOUSLY AND IN INCREASING AMOUNTS THROUGHOUT THE MENSTRUAL CYCLE

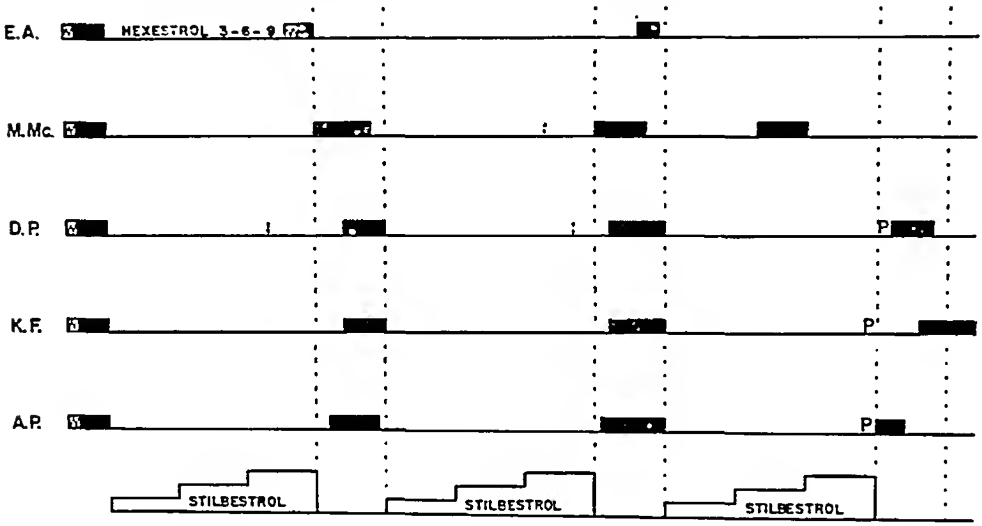

FIG. 3. Graphic representation of prolonged treatment with stilbestrol in patients with dysmenorrhea. The black bar with the zigzag represents a painful menstrual period, whereas, the other periods were painless. The daily dose of stilbestrol was increased at intervals of ten days from 1 to 2, and then to 3 mg. orally. The medication was withheld for ten days before the next series was offered. Proliferative endometrium (P) was obtained in three instances.

the onset of bleeding. One patient also experienced episodes of nausea and headaches during the treatment. A patient not shown on the chart discontinued therapy because of nausea. For those patients who tolerate the drug, stilbestrol can be given orally in such a manner that dysmenorrhea may be completely relieved for as long as three successive months. It has not been continued longer, and consequently we have no data as to whether the endometrial hyperplasia and profuse bleeding reported by Sturgis and Meigs might have resulted from longer use.

From these experiments and those of Sturgis and co-workers, it is apparent that prolonged or continuous treatment with estrogen will prevent ovulation and eliminate the luteal phase from the cycle.

LATE TREATMENT

It has been reported by Nelson and associates (7, 8) that estrogen maintained functional corpora lutea in the rat and Heckel and Allen (9) made similar observations in the rabbit. Westman (5) administered estrogen to

THE EFFECT OF ESTROGEN GIVEN IN THE MIDDLE OR THE LUTEAL PHASE OF THE CYCLE

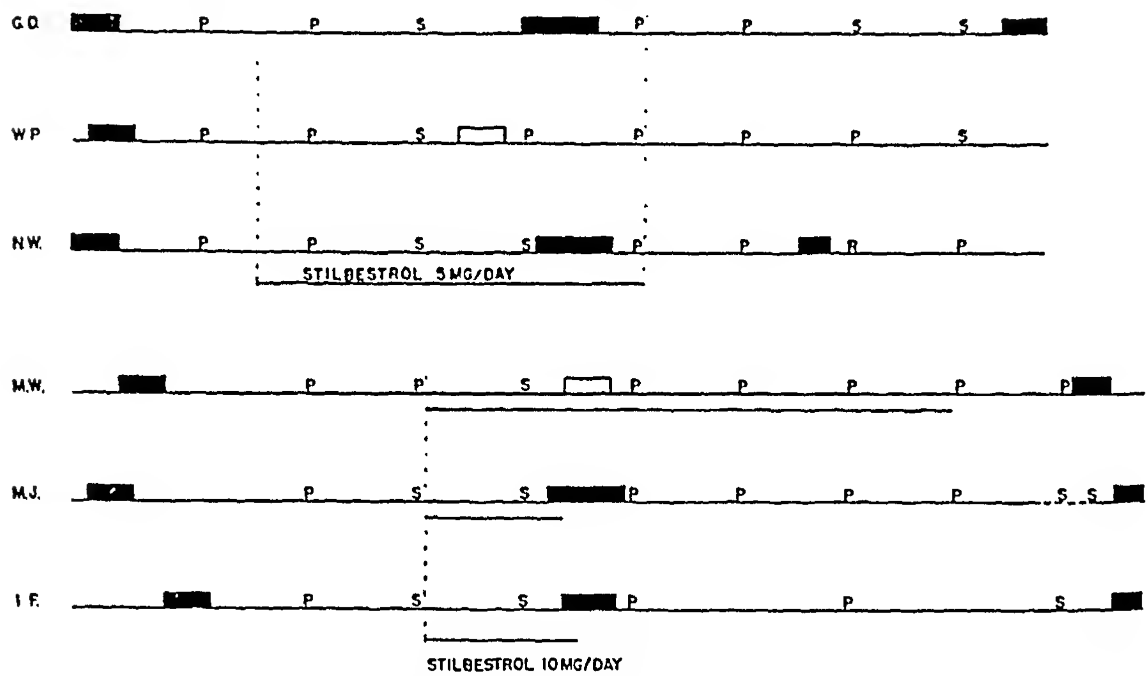

FIG. 4. Graphic representation of the menstrual intervals and endometrial findings in patients given stilbestrol through the luteal phase of the cycle. The horizontal lines represent the duration of treatment. The letters denote the condition of the endometrium, P for proliferative and S for secretory. The two open bars represent probable menstrual periods in patients who could not give reliable menstrual dates; the change in the endometrium indicated an unrecorded menstruation.

two women and then, on the basis of one premenstrual biopsy and one biopsy taken nine weeks after the previous period (in a patient with irregular cycles), presumed he had maintained luteal function in a manner similar to that demonstrated in the experimental animal.

Three women were given 10 mg. of stilbestrol daily starting in the postovulatory (luteal) phase (Fig. 4). There was no augmentation or prolongation of the luteal phase in these patients. One of the subjects (M. W.) was a mental patient and unable to give a menstrual history. The change from secretory to proliferative pattern in the endometrium indicated that the

patient had bled even though this fact was not recorded. It is interesting in her case that prolonging the estrogen treatment for an additional three weeks resulted in an anovulatory menstruation in the subsequent cycle; whereas, in the other two women the estrogen therapy was stopped with the onset of bleeding and the subsequent cycle was ovulatory.

In order to determine if earlier or more prolonged administration of estrogen would be effective, three women were given 5 mg. of stilbestrol daily starting about the tenth day of the cycle (Fig. 4). There was no increase in the length of the cycle and progestational changes in the endometrium were normal in degree and duration. These observations offer no evidence that the human corpus luteum is affected in any way by estrogen. On the basis of these observations it remains to be demonstrated whether estrogen will maintain functional corpora lutea in women as has been demonstrated in rats and rabbits.

DISCUSSION

From the foregoing observations, it is evident that a single dose of estrogen given early in the cycle may delay ovulation for a period of seven to ten days. When the estrogen is given over a period of time (fourteen to thirty days) ovulation is completely suppressed. However, if estrogen treatment is started after the follicle has matured or ovulation has occurred, it has no effect on the functional span of the corpus luteum. Thus the effect of estrogen varies with the dosage, the timing and the duration of treatment.

These experiments afford no evidence as to the mechanism whereby the estrogen exerts its effect. It is not possible to determine whether the estrogen may have some direct effect on the ovary or whether its effect is through the stimulation or suppression of gonadotropic activity of the anterior pituitary. Sturgis and Meigs (2) suggested that the estrogen "repressed" pituitary function. There is also the possibility that a single dose of estrogen early in the cycle can release hormone from the anterior pituitary before the Graafian follicle is mature enough to respond. This discharge of gonadotropin following estrogen was suggested by D'Amour (10). Experiments testing this hypothesis are reported elsewhere (11). Premature discharge of gonadotropin and a latent period for recovery of normal pituitary potency could account for the prolonged cycles when estrogen is given soon after a menstrual period. Continuous or prolonged estrogen treatment would delay restoration of pituitary hormone content so that anovulatory cycles would seem a logical result.

It has been shown experimentally that progesterone protects the pituitary from depleting its hormone content under the influence of estrogen. The lack of any effect of estrogen during the luteal phase may be due

to the presence of progesterone. The validity of this argument in human physiology should be tested by giving progesterone with the single dose of estrogen early in the cycle to determine whether the progesterone would negate the estrogen effect.

SUMMARY

The effect of estrogen on the human ovarian cycle varies with the mode of administration.

1. A single large dose (10 to 20 mg. of stilbestrol orally) given early in the cycle will delay ovulation for about ten days and increase the length of a cycle similarly. This schedule delays but does not inhibit or alter the normal sequence of ovulation and corpus luteum formation.

2. Estrogen (stilbestrol) given continuously for thirty days in daily doses increasing from 1 mg. to 3 mg. will completely inhibit ovulation. This can be repeated for at least three successive cycles without producing complications.

3. Estrogen (stilbestrol) started late in the cycle has no effect on the appearance, or on the duration of the luteal phase of the cycle. Stilbestrol given orally in doses of 10 mg. daily has not maintained functional corpora lutea in women.

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EFFECT OF LOW DOSAGE ROENTGEN RADIATION ON THE GONADOTROPIC FUNCTION OF THE HYPOPHYSIS OF THE MATURE AND IMMATURE FEMALE ALBINO RAT

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I. INTRODUCTION

In view of the wide interest and frequent use of low dosage roentgen therapy in the treatment of functional endocrine dyscrasias, and the many controversial theories concerning its effect on glandular function, it has seemed important to study the action of radiation on the normal pituitary of the experimental animal as manifested in its sexual system.

Although thirty-two years have elapsed since van de Velde (1) first used low dosage roentgen therapy as a therapeutic agent in the treatment of functional menstrual disorders and sterility, its mode of action on the endocrine glands is still very poorly understood. Many theories have been proposed to explain the good results reported clinically in hypofunctional endocrine conditions. All these theories can be divided into two main groups: 1) stimulation; 2) selective destructive action. Schwarz (2) believes a direct stimulation of glandular function is produced by small doses of x-rays, through an alteration of biochemical factors such as an increase of reversible cell permeability, or a rearrangement of electrons, atoms, and molecules, to increase the degree of cellular activity without the production of detectable changes in cellular structure. He also feels that an indirect stimulation may be mediated through the production of an active hyperemia in the irradiated gland, causing an increased blood supply, and resulting in an increased metabolic activity of the irradiated cells. Mazer and his co-workers (3, 4, 5, 6), Kaplan (7, 8) and several others (9, 10) refer to this theory of stimulation as the probable explanation of the 45 to 70 per cent clinical cures of various menstrual dysfunctions noted in their series of cases following radiation of the pituitary, the pituitary and ovaries or the ovaries. Thaler (11), Desjardins (12, 13), Tamis (14) and numerous other workers (15, 16, 17) refuse to accept this theory, and believe that x-rays, regardless of the smallness of the dose, always produce degenerative

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and destructive changes in the irradiated cells. They prefer to explain this apparent stimulation on the basis of a release of some check on the normal cyclic activity of the ovary, such as the destruction of a large Graafian follicle which is preventing the growth and maturation of other primordial follicles, or the destruction of a persistent corpus luteum which is suppressing menstruation. This is based on the experimental work of Van Pee and Simon (18), who irradiated the ovaries of mature rabbits and dogs and demonstrated a selective sensitivity of the various structures in the ovary to x-rays. The large maturing follicle was found to be the most sensitive and the corpus luteum the least sensitive, due in all likelihood to the less mature and more actively growing granulosa cells of the follicle.

Cystic ovaries have long been known to cause delay in menstruation Robinson (19) and Stein and his co-workers (20, 21, 22) have reported several cases of bilateral cystic ovaries associated with amenorrhea and sterility which were cured by surgical removal of the cystic portions of the ovary.

Good results have been reported by Steinhardt (23), Werner (24, 25) and others (26) following radiation of the pituitary alone in cases of amenorrhea and dysmenorrhea. Proponents of the theory that x-rays always have a destructive or inhibitory effect on cellular activity argue that the menstrual disorder in such cases probably was due to a hypersecretion of follicular stimulating hormone by the anterior lobe cells of the hypophysis, and that the reported cures resulted from an inhibition of these hyperfunctioning cells. Zondek's (27) experimental work has shown that amenorrhea and uterine bleeding are due in many instances to hypersecretion of the anterior lobe of the pituitary. If this condition persists for a long time histological evidence appears in the ovaries in the form of multiple cysts.

Newell and Pettit (28) reported a series of patients who were treated for various functional gynecological disorders. The blood of thirteen patients who received radiation of the pituitary was assayed for anterior pituitary sex hormone both before and after x-ray treatment. Ten patients showed an excess of anterior pituitary hormone in their blood before treatment. This excess hormone is reported to have disappeared after x-ray treatment in seven but persisted in the other three patients. In three patients the anterior pituitary hormone level in the blood was considered to be normal before treatment and did not change following pituitary radiation. Though the number of patients in which such studies were done is very small, they felt that there was a definite indication that this hormonal function of the pituitary gland was influenced (diminished) by even small doses of x-rays.

Thus the theories of the mode of action of small doses of x-rays on the glandular function are numerous, widely divergent, and contradictory. It

was decided therefore to study the effect of low dosage radiation on the normal hypophysis of the albino rat: 1) in order to determine whether there was any evidence of an alteration in glandular function, and 2) if any effect was demonstrated, by what mechanism it was produced.

II. REVIEW OF EXPERIMENTAL LITERATURE

The first studies of the effects of radiation of the pituitary in animals were made by Brunner (29) in 1920. He irradiated the entire head of young cats and dogs and reported some histological changes in the pituitary following fairly large doses of x-rays as well as slight histological changes in the nervous tissue and some suppression of growth. He stated that the pituitary was only slightly sensitive to x-rays. Fraenkel and Geller (30) in 1921 observed weight loss, growth retardation, and hypoplasia genitalis following radiation of the hypophysis of young female rabbits. In 1922, Rahm (31) irradiated the hypophyseal regions of young rabbits and observed a stimulation of growth with small doses and a cessation of growth with large doses. Podljaschuk (32, 33) in 1927 and 1928, using young and mature rabbits and dogs, and Mahnert, (34) using infantile mice, observed an inhibition of body growth and underdevelopment of sex organs. Epifanio and Cola (35) found that a 25 per cent erythema dose directed to the hypophysis of young rabbits accelerated growth, but that an erythema dose caused suppression of growth. With the erythema dose there were demonstrable histological changes in the pituitary, the acidophiles being the most affected. Selle, Westra, and Johnson (36) using doses as high as 3000 r directed to the pituitary of depancreatized dogs were unable to ameliorate their diabetes. Histologically, the hypophysis showed congestion and degeneration; degenerative changes were found in the nervous tissue of the brain.

Lacassagne (37) introduced radon and radium needles into the hypophyseal glands of rabbits and found partial to complete destruction of the gland after long periods of exposure. Associated with the pituitary necrosis he observed loss of sex drive and gonadal atrophy. He found that if one-third of the gland remained, this was sufficient to produce a normal function of the ovary, and concluded that the pituitary was very radioresistant.

Fehr (38) studied the effects of both massive and fractionated doses of roentgen radiation over the pituitary regions of mature rabbits. He administered doses up to 10,160 r to adult rabbits in amounts of 600 r over a period of two to three months, but did not demonstrate any physiological effect, and no histological changes were found in the pituitaries when the animals were sacrificed four to six weeks later. With single massive doses of 2860 r there was a demonstrable weight loss, diminution of sex drive, and the ovaries and uterus were juvenile in appearance. He calculated the tissue

dose to the pituitary to be about 80 per cent of the skin dose and concluded that the rabbit pituitary was very radioresistant and could not be influenced by ordinary doses of x-rays.

The results observed by many of these early workers were varied and often contradictory, but the majority of the studies seem to indicate that the hypophysis of these various laboratory animals was relatively radioresistant. However, it must be remembered that much of this early work was done with x-ray equipment that was inefficient by modern standards, and since there was no accepted dose standardization, there was often no real basis for comparison of the results of one worker with those of another.

Lawrence, Nelson, and Wilson (39) studied the effects of radiation of the hypophysis in immature albino rats using factors of 180 kv., 25 ma., filter 0.5 mm. Cu and 1 mm. Al, 50 cm. distance, and a 1 cm. circular portal. With doses of 513 (air) r repeated at three or four sittings about five days apart, there was only a slight inhibition of gain in weight, but when two treatments of 1040 (air) r were given at a similar interval, there was a definite suppression of growth and ovarian activity. The pituitaries, ovaries, thyroids, and adrenals of irradiated animals sacrificed eighteen to twenty-two days after x-ray therapy were definitely decreased in size. Histologically, there was a scarcity of acidophile cells in the hypophysis and the basophile cells, though normal in number, showed some distinct clumping of the granules and frequent pyknotic nuclei. This damage, however, was not permanent as there was a gradual return of the hypophyseal structure and function to normal in animals sacrificed two to three months after treatment. They conclude that although the pituitary glands of young albino rats are relatively radioresistant, nevertheless they can be influenced by x-rays.

Because of the numerous references to cures of sterility in women by means of "stimulating" doses of x-rays, Hartman and Smith (40) in 1938 made a study of the effects of small single doses of from 60 to 400 (air) r on 13 sterile female rhesus monkeys, using factors of 200 kv., 20 ma., filter 0.5 mm. Cu and 1 mm. Al, 50 cm. distance, 4 by 5 cm. field. Although their findings were somewhat indefinite, they concluded that x-rays in the doses used had no effect on the pituitary of these animals. Kotz, Edward, and Parker (41) irradiated the hypophyses of 20 adult female rats with single doses of 200 to 500 r using factors of 200 kv., 25 ma., filter 0.5 mm. Cu and 1 mm. Al, 50 cm. distance. The animals were sacrificed from three to seven weeks after roentgen treatment and histological studies were made of the pituitaries, ovaries, thyroids, and adrenal glands. No degenerative changes were noted in these endocrine glands. They concluded that radiation of the pituitary in the doses used had no harmful effects.

Denniston (42) in 1942 irradiated the pituitaries of adult and immature

ground squirrels and albino rats. He used factors of 200 kv., 4 ma., filter 0.5 mm. Cu and 1 mm. Al, 60 cm. distance. Doses varying from 7 to 140 r were used in immature ground squirrels, and at autopsy three to twelve weeks after treatment, he found the pituitary and sexual organs of the treated animals in no way different from those of the controls. Most of the animals were treated with doses ranging from 500 to 4000 r. He reached the following conclusions: 1) The pituitary is extremely resistant to x-rays. 2) No good evidence of a "stimulating dose" has been found from these studies. 3) A differential susceptibility of the anterior lobe cells is demonstrated; the alpha cells lose their characteristic granules, the cytoplasm shrinks, and there is an increase in size of their nucleoli; the beta cells and chromophobes are apparently unaffected within the dosage of 500 to 4000 (air) r used in the ground squirrel. 4) The growth rate of young rats is reduced 50 per cent following radiation of the pituitary with 1000 (air) r or more.

Hodes and Israel (43) treated adult female rabbits with doses of 50 to 80 (air) r to both the ovaries and pituitary using factors of 200 kv., 15 ma., half-value layer 1 mm. Cu, distance 50 cm., and could demonstrate no evidence of ovulatory stimulation following radiation.

While our study was in progress, Baidins, Claesson and Westman (44) were also studying the effects of radiation of the pituitary in infantile rats using factors of 105 kv., 10 ma., filter 1 mm. Al and 165 kv., 7 ma., with 0.5 mm. Cu filtration. Their results were reported in 1946. With single treatments of 2 to 45 r, they noted an increase in the size and weight of the pituitaries of the treated animals sacrificed from eleven to fourteen weeks after treatment. The only histological change was a slight hyperemia of the pituitaries. Similar observations were made after single doses of 50 to 500 r but in these rats the hyperemia was more marked and there was a greater increase in the size and weight of the pituitaries. The ovaries of the treated rats were in no way different from the controls. Even though the increase in size and weight of the pituitary seemed to indicate a stimulating effect of radiation on the pituitary, they were unable to demonstrate any evidence of an increase in function of either the pituitary or ovarian cells. They therefore concluded that it was impossible to influence the pituitary gland of the white female infantile rat by small doses of x-rays.

Though a review of these recent experimental studies with low dosage radiation of the pituitary (doses of 400 (air) r or less) has shown no histological effects on the sexual system of animals, the immediate and latent physiological effects were seldom carefully observed. In view of the importance of this highly controversial problem, we thought that further investigation was indicated using methods designed chiefly to study and evaluate any physiological effect that might be produced.

III. MATERIALS AND METHODS

Ninety *sexually mature* female Wistar albino rats were used in the first part of this study. The Wistar albino rat was chosen as the test animal since it has a short oestrus cycle and experimental methods have been established for determining the various phases of its oestrus cycle. The length of its cycle is 4 or 5 days and there are five different stages; each is characterized by periodic changes in the epithelium of the uterus and vagina. The various phases of the cycle can also be determined from the rat's running activity. There is a marked rise in the activity of the rat on the first day of the cycle, day of heat, and period of ovulation. The activity then decreases and remains low throughout the next two days and then begins to rise slowly on the fourth day with a marked peak again on the first day of the next cycle.

Farris (45) has studied a large number of Wistar female albino rats over long periods of time and has found the activity records more satisfactory than vaginal smears for determination of the different stages of the cycle.

The signs of heat in the rat are as follows:

1. Ear quivering (EQ) elicited by stroking gently the head or back.
2. Lordosis or copulation response (CR) produced by digital stimulation of the pelvic region.

Whenever the symbol "EQCR"[1] is used it indicates the presence of the combined response to "EQ" and "CR," as referred to above.

When in heat the rat is nervous, apprehensive, darting about, and on being touched, braces herself. This period of heat usually lasts from eight to twelve hours, and it is only during this time that she will accept the male.

The rats were kept in a special colony room at The Wistar Institute. The colony room was darkened completely from 8 a.m. to 8 p.m. Four 100 watt lights were turned on automatically at 8 p.m. and remained on until 8 a.m. By this method the rats display more regular oestrus. On the first day of the cycle heat occurs about 9 a.m., thus bringing it within our working day.

Activity determinations were made by keeping the rats in cages equipped with turntables connected to electrical counters which recorded each revolution of the turntables. Activity records were kept on all the rats, and they were checked for signs of heat on the day of oestrus.

Roentgen treatments were given at 7.30 a.m. on the second day of the cycle in all of the irradiated rats, except in five which were treated at 7.30 a.m. on the third day. No rats were treated unless signs of heat had been elicited on the preceding day of oestrus and were absent on the morning

[1] Abbreviation used in tables in text.

of the second day. After treatment they were returned immediately to their cages and checked at half-hour intervals for signs of heat, for a period of three hours following radiation of the pituitary. For each two treated rats, one control was taken out of its cage and handled similarly to the irradiated rats, but received no radiation.

Six treated rats and seven of the day-two control animals were sacrificed and studies made of the pituitaries, ovaries, and uteri. These organs were then compared with the organs of six untreated rats sacrificed on day-one, the normal day of oestrus.

Twenty-two *immature* female Wistar albino rats twenty-eight days in age were used in the second part of this study. Vaginal opening was taken as the sign of sexual maturity. Eight of these rats were treated and followed daily until vaginal opening occurred. Two others were used as controls. An additional eight were treated, and together with four controls, were sacrificed when forty days of age and pituitary, ovarian, and uterine weight determinations were made at this time.

The following *method of treatment* was used throughout the experiment: The rats were treated with an intermediate therapy unit using factors of 135 kb., 8 ma., inherent filtration 1 mm. Al with 2 mm. Al added giving a half-value layer of 4 mm. Al. A circular cone 1.8 cm. in diameter was used with a distance of 24 cm. from the target to skin surface. The roentgen output at the end of the cone was 90 (air) r per minute. The pituitary gland of the rat is located at an approximate depth of 1 cm. from the skin surface. Depth dose measurements were determined at 1 cm. using a fiberwood phantom and then expressed in terms of the air roentgens as measured at the skin surface. This gave a depth dose measurement at 1 cm. equal to 76 per cent of the air roentgens delivered at the skin surface. The doses are given in terms of air roentgens throughout this report and can be reduced to tissue roentgens by multiplying by the above percentage factor.

The rat was immobilized by wrapping it tightly in a cotton cloth and then molding a lead cage about the body so that only its head was exposed. A lead collar was slipped about the neck. The treatment cone was then brought down on the head and centered over the pituitary region. Treatment sometimes had to be interrupted to re-position the rat's head in case of movement, but due to the relatively short treatment times this difficulty was minimized.

IV. RESULTS

(A) EFFECTS OF RADIATION OF THE PITUITARY ON THE SEXUAL ACTIVITY OF THE MATURE FEMALE RAT

1. *Single Dose.* Fifty-nine female rats varying in age from seventy-seven to two hundred and seventy days were used. All had regular four or five day

TABLE 1. RESULTS FOLLOWING SINGLE DOSES OF ROENTGEN RADIATION DIRECTED TO THE PITUITARY OF THIRTY-NINE SEXUALLY MATURE ALBINO FEMALE RATS

RAT			DOSAGE	EFFECTS OF TREATMENT	
Identification number	Age in days	Day of oestrus cycle	In (air) roentgens	EQCR* response	Mating
77′	240	2	10	none	no
68	245	2	10	none	no
62	270	2	20	none	no
67′	83	2	25	none	no
302	142	2	25	none	no
60′	233	2	50	none	no
219	128	2	50	none	no
208	136	2	50	EQCR	mating
217	93	2	50	EQCR	mating
106	183	2	50	EQCR	no
202	138	2	100	EQCR	mating
220′	189	2	100	EQCR	mating
303	133	2	100	EQCR	mating
73	212	2	100	none	no
70′	190	2	100	none	no
74	237	2	100	none	no
203	133	2	150	EQCR	mating
76[2]	106	2	150	none	no
75′	206	2	150	none	no
66	141	2	200	none	no
61	245	2	200	none	no
102′	77	2	200	none	no
103′	77	2	200	none	no
105	182	2	200	none	no
206	144	2	200	none	no
69′	83	2	200	none	no
301	131	2	200	none	no
97′	106	2	200	EQCR	no
78	217	2	200	EQCR	mating
65	240	2	200	EQCR	mating
90	234	2	200	EQCR	no
67′	245	2	200	EQCR	no
95′	126	2	200	EQCR	no
69	343	2	200	EQCR	no
72′	106	3	150	none	no
64	213	3	100	none	no
79	235	3	200	none	no
71[2]	149	3	200	none	no
98	247	3	200	none	no

* Symbol EQCR used in this table indicates ear quivering and copulation response.

cycles as shown by their running activity. Single doses of radiation to the pituitary varying in amount from 10 to 200 r were given to thirty-nine of these rats as shown in Table 1; twenty were used as controls.

Thirty-four of these thirty-nine rats were treated on the morning of the second day of the cycle. None of five rats of this group receiving less than 50 r showed any evident response to the radiation treatment. However, three of five rats given 50 r; three of six rats given 100 r; one of three rats

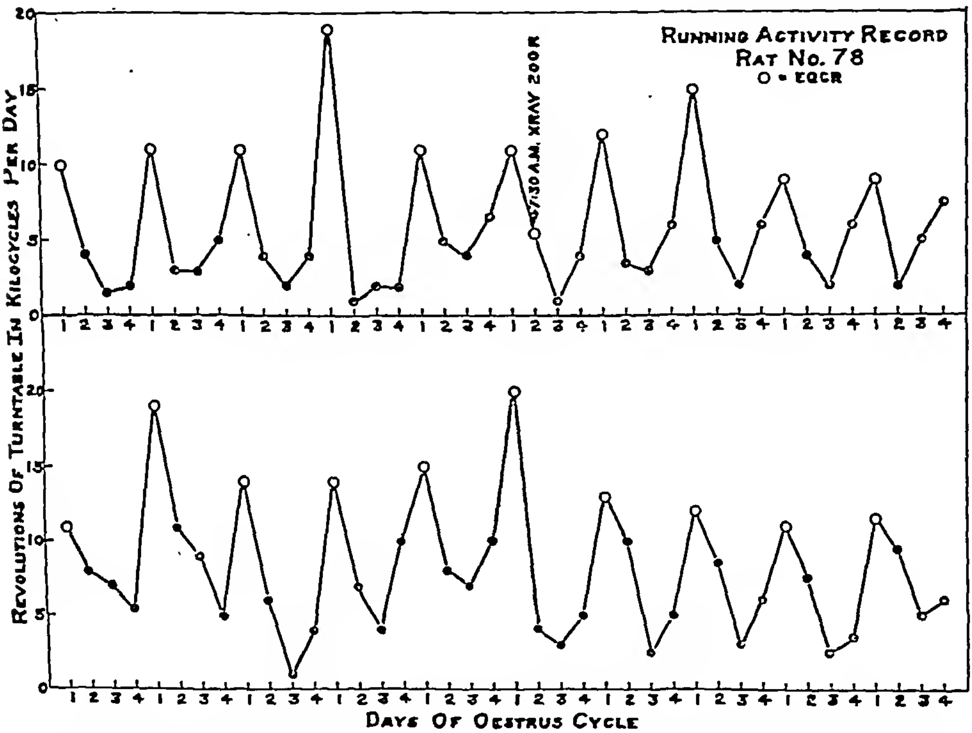

FIG. 1. Typical activity record of one rat (No. 78) in whom an EQCR response was noted on day-two of the oestrus cycle following a single treatment of 200 roentgens to the pituitary.

given 150 r; and seven of fifteen rats given 200 r showed signs of heat from one to two hours after treatment, which persisted for four to twelve hours. These findings were similar to those observed on the normal day of heat, which day is day-one of the cycle, differing only in that the running activity did not increase but tended to drop off, as in the untreated rat. Thus, a reappearance of heat signs was observed in fourteen (41.2 per cent) of these thirty-four rats following radiation, while twenty appeared to be unaffected by the treatment. The activity records of these rats were followed for 20 to 30 cycles after treatment and no change was noted in the periodi-

city of their cycles. A typical activity record of one of these rats in whom an EQCR response was noted following a treatment of 200 r is shown in Figure 1. All of the fourteen rats in whom an EQCR response was obtained on day-two following pituitary radiation were checked for mating and eight or 57.3 per cent accepted the male and two pregnancies resulted.

The remaining five of the thirty-nine rats were treated on the morning of day-three of the cycle with doses varying from 100 to 200 r, but none of them appeared to be affected in any way by the treatment.

TABLE 2. RESULTS FOLLOWING SMALL SERIAL DOSES OF ROENTGEN RADIATION DIRECTED TO THE PITUITARY OF TWENTY-THREE SEXUALLY MATURE ALBINO FEMALE RATS ON THE SECOND DAY OF EACH CONSECUTIVE CYCLE

Number of rats treated	Doses per treatment in (air) roentgens	Number of rats showing an EQCR response to TREATMENT 1st	2nd	3rd	4th	5th	6th	7th	8th	Number of rats that failed to show ECQR response after 15 treatments
9	5	3	2	1						3
6	10	1							1	4
1	15	1								—
6	20	3				1				2
1	25	—	—	1						—
Total 23		8	2	2		1			1	9

In the group of fifty-nine rats the twenty used as controls were handled in a similar manner to the thirty-nine treated rats, but no radiation was given. This was done to eliminate the possibility that handling alone might account for the EQCR response noted following treatment. None of the twenty controls showed any signs of heat on day-two or change in the regularity of their cycles as a result of this handling.

2. *Serial Doses.* A group of thirty-one sexually mature female rats was used to study the effect of serial treatments to the pituitary. Twenty-three of these received radiation and eight were used as controls. Radiation was given in doses from 5 to 25 r on the second day of each cycle. These same doses were repeated for as many as 10 to 15 consecutive cycles to determine whether there was any prolonged effect. The largest total treatment received by any one rat was 300 r. A summary of the EQCR response obtained by this method of serial radiation of the pituitary is shown in Table 2.

Three of the nine rats receiving 5 r responded to the initial treatment, two responded to the second treatment, one to the third treatment, and three were unaffected after fifteen treatments.

One of six rats given 10 r responded to the first treatment, one responded after eight treatments, and four were unaffected after fifteen treatments.

One rat receiving 15 r responded to this initial dosage.

Three of six rats given 20 r responded to a single treatment, one responded after five such treatments, and two were unaffected after fifteen treatments.

One rat receiving 25 r responded after the third of such treatments.

TABLE 3. PITUITARY, OVARIAN, AND UTERINE WEIGHT DETERMINATIONS IN SEXUALLY MATURE ALBINO FEMALE RATS FOLLOWING RADIATION OF THE PITUITARY

Rat identification *number*	Pit. wt. in mg.	Pit. wt./ B.W. $\times 10^{-4}$	Pit. wt./ B.L. $\times 10^{-4}$	Ov. wt. in mg.	Ov. wt./ B.W. $\times 10^{-4}$	Ov. wt./ B.L. $\times 10^{-4}$	Ut. wt. in mg.	Ut. wt./ B.W. $\times 10^{-4}$	Ut. wt./ B.L. $\times 10^{-4}$	Treatment in (air)
CONTROL RATS (DAY-TWO)										
104	11.7	.540	.585	108.1	4.66	5.24	505.6	21.8	24.4	
68	9.6	.424	.480	75.0	3.32	3.75	317.6	13.8	15.6	
73	10.4	.507	.525	83.1	4.05	4.20	359.3	17.5	18.1	
102	12.3	.545	.600	105.7	4.67	5.15	632.8	28.0	30.9	
75	12.0	.629	.609	110.5	5.79	5.61	604.5	31.6	30.7	
78	14.1	.572	.678	99.7	4.03	4.80	532.7	21.5	25.6	
77	12.5	.553	.598	87.2	3.86	4.18	555.9	22.9	26.2	
Total:	82.6	3.770	4.075	669.3	30.38	32.93	3508.4	157.1	171.5	
Average:	11.8	.538	.582	95.6	4.34	4.70	501.2	22.4	24.5	
TREATED RATS (DAY-TWO)										
215	16.5	.665	.774	106.2	4.28	4.98	607.5	24.5	28.5	200r
220	15.6	.596	.712	111.2	4.35	5.18	613.9	24.1	28.6	100r
218	13.0	.552	.617	120.6	5.00	5.65	529.8	21.9	24.8	10r
205	14.7	.596	.688	70.7	3.02	3.39	682.9	29.2	32.6	10r
214	15.2	.630	.730	79.6	3.06	3.76	744.0	28.2	35.1	5r
204	14.8	.641	.711	104.7	4.52	5.04	511.3	22.7	24.6	10r
Total:	89.8	3.680	4.232	593.0	24.23	28.00	3689.4	150.6	174.2	
Average:	14.9	.613	.705	98.8	4.04	4.66	614.9	25.1	29.0	
UNTREATED RATS IN OESTRUS (DAY-ONE)										
71	13.4	.556	.641	101.9	4.61	4.94	828.4	34.4	39.6	
211	14.1	.576	.659	112.3	4.54	5.20	1143.1	46.6	53.3	
305	12.5	.558	.601	107.6	4.81	5.18	1112.3	49.6	53.4	
200	12.5	.546	.616	101.4	4.45	5.03	1178.7	51.5	58.1	
95	14.3	.622	.697	103.9	4.51	5.06	1084.5	47.1	52.6	
74	13.6	.670	.687	82.9	4.08	4.19	971.1	47.4	49.0	
Total:	80.4	3.528	3.901	610.0	27.00	29.60	6318.1	276.6	306.0	
Average:	13.4	.588	.650	101.6	4.50	4.93	1053.0	46.1	51.0	

Pit. Wt. = Pituitary weight.
Ov. Wt. = Ovarian weight.
Ut. Wt. = Uterine weight.

B.L. = Body length.
B.W. = Body weight.

Seven of the fourteen rats in whom an EQCR response was observed following serial treatments accepted the male and two pregnancies and one pseudopregnancy resulted.

The EQCR response in these rats following serial treatments was similar to that noted on the normal day of heat except that their running activity was not stimulated. The periodicity of their cycles was unchanged. Most of these rats after showing a response to the radiation of the pituitary continued to show a similar response to several subsequent treatments.

TABLE 4. AVERAGE DIFFERENCE IN ORGAN WEIGHTS BETWEEN UNTREATED FEMALE RATS IN NORMAL OESTRUS AND UNTREATED DAY-TWO CONTROLS

Average	Pit. wt. in mg.	Pit. wt. B.W. $\times 10^{-4}$	Pit. wt. B.L. $\times 10^{-4}$	Ov. wt. in mg.	Ov. wt./ B.W. $\times 10^{-4}$	Ov. wt./ B.L. $\times 10^{-4}$	Ut. wt. in mg.	Ut. wt./ B.W. $\times 10^{-4}$	Ut. wt./ B.L. $\times 10^{-4}$
In rats in normal oestrus	13.4	.588	.630	101.6	4.50	4.93	1053.0	46.10	51.00
In day-two control rats	11.8	.538	.582	95.6	4.34	4.70	501.2	22.40	24.50
Difference in weight +	1.6	.05	.068	6.0	0.16	0.23	551.8	23.70	26.55
Difference in per cent +	13.5	9.20	11.60	6.2	3.60	4.90	110.0	105.00	108.00

+ = increase.

The eight controls of this group of thirty-one rats received no radiation and they showed no signs of heat on day-two as the result of handling.

3. *Gross Anatomical Studies.* Six of the rats who showed an EQCR response following radiation treatment on day-two were sacrificed six hours later together with seven day-two controls. The pituitary, ovaries, and uterus were removed and weight determinations made. These were then compared with similar studies made on six untreated rats sacrificed on day-one, the normal day of oestrus. The ratio of these organ weights to that of the whole body weight and body length of the rat were calculated for all three of these groups, to make allowances for differences in the weight and size of the animals. The weight determinations and the average for the three groups are shown in Table 3.

The average percentage differences in the organ weights of the untreated rats sacrificed on the day of oestrus as compared to the untreated control rats killed on day-two is shown in Table 4. The average pituitary weight was 13.5 per cent greater in the rats in normal oestrus, but this difference is reduced to 9.2 per cent due to the average greater body weight in this group. There is no significant difference in ovarian weights when the averages for the two groups are compared. The average uterine weight of

the rats in normal oestrus however, was 110 per cent above that in the two-day controls.

The average percentage differences in the organ weights of the treated rats as compared to the untreated control rats sacrificed on day-two are shown in Table 5. The average pituitary weight was 26.2 per cent greater in the treated rats when compared to that of the controls but this difference is reduced to 13.9 per cent when body weight variations are considered. The average ovarian weight was essentially the same in the two groups.

TABLE 5. THE AVERAGE DIFFERENCE IN ORGAN WEIGHTS BETWEEN FEMALE RATS TREATED ON DAY-TWO AND THE DAY-TWO CONTROLS

Average	Pit. wt. in mg.	Pit. wt./B.W. $\times 10^{-4}$	Pit. wt./B.L. $\times 10^{-4}$	Ov. wt. in mg.	Ov. wt./B.W. $\times 10^{-4}$	Ov. wt./B.L. $\times 10^{-4}$	Ut. wt. in mg.	Ut. wt./B.W. $\times 10^{-4}$	Ut. wt./B.L. $\times 10^{-4}$
In day-two treated rats	14.9	.613	.705	98.8	4.04	4.66	614.9	25.10	29.00
In day-two control rats	11.8	.538	.582	95.6	4.34	4.70	501.2	22.40	24.50
Difference in weight +	3.1	.075	.123	3.2	—	—	113.7	2.70	4.50
Difference in per cent +	26.2	13.90	21.10	3.3	—	—	22.6	12.00	18.40

+ =increase.

The average uterine weight was 22.6 per cent greater in the treated rats but this difference is reduced to 12 per cent on considering body weight and body length variations.

(B) EFFECTS OF RADIATION OF THE PITUITARY ON THE DEVELOPMENT OF THE SEXUAL SYSTEM OF THE IMMATURE FEMALE RAT

1. *Single and Serial Doses.* Twenty-two immature female Wistar albino rats twenty-eight days in age were used in this part of the study.

Of the first ten rats studied, eight were treated by radiation of the pituitary in small doses varying from 5 to 50 r, and two were used as controls. A pair of rats was given doses of 5 r, one receiving only a single treatment whereas the other received five treatments on successive days. Similarly the other three pairs were treated with single and with five daily doses of 10, 20, and 50 roentgens, respectively, as shown in Table 6. The maximum dose received by any one rat was only 250 r. These rats were then followed daily until vaginal opening occurred. One died of unknown cause before the study was completed. Vaginal opening occurred at ages ranging from fifty-three to sixty-five days in both the controls and treated rats. This is

within the normal limits for the onset of sexual maturity as manifested by vaginal opening, according to the work of Long and Evans (46).

Eight of the remaining twelve immature rats were then similarly treated with single and five daily treatments of 10, 20, 50, and 100 roentegns and the other four were used as controls. These animals were sacrificed twelve days after radiation of the pituitary and pituitary, ovarian, and uterine weight determinations were made as was done with the sexually-mature rats. There was no significant organ weight difference between these

TABLE 6. RESULTS OF RADIATION OF THE PITUITARY ON THE ONSET OF PUBERTY IN 28-DAY-OLD FEMALE RATS AS DETERMINED BY THE TIME OF VAGINAL OPENING

Rat identification number	Dose administered per treatment in (air) roentgens	Total number of successive daily treatments	Age at vaginal opening in days
0	5	1	53
1	5	5	63
2	10	1	53
3	10	5	58
4	20	1	59
5	20	5	died
6	50	1	65
7	50	5	56
8	control	1 } handling	59
9	control	5 } only	57

eight treated rats and their four controls. Grossly the appearance of these treated organs was in no way different from the control untreated organs.

V. DISCUSSION

In a study of the effects of various pituitary and ovarian hormone preparations on the rhythm of the oestrus cycle and running activity of the rat, Farris (47) was able to show that the appearance of the clinical signs of heat was dependent upon the secretion of follicle-stimulating hormone by the pituitary and the resultant stimulation of estrogen production in the ovary. The increased running, however, was largely due to estrogen. Urine extracts high in their luteinizing effect when injected into the normal rat on the day of peak activity caused a sudden marked drop in its activity and disappearance of heat signs. Similarly injections of progesterone caused a decrease in activity but this was not as marked as that following injections of pituitary luteinizing hormone. Thus he was able to correlate the running activity of the rat with the various hormone preparations believed to be involved in the production of the oestrus cycle.

Signs of heat, mating, and an increase in running are normally observed during the period of oestrus, on the first day of the oestrus cycle. This period of oestrus lasts from eight to twelve hours. Farris, (45) in a previous study of a large number of rats, has observed only occasionally these physiological signs of heat on the second day of the cycle or the metoestrus period.

None of our twenty-eight controls in the sexually mature group handled similarly to the irradiated rats in this group showed any signs of heat or change in the regularity of their cycles as the result of handling.

Thus, the reappearance of heat signs on day-two of the oestrus cycle, from one to two hours after single or multiple doses of radiation of the pituitary in twenty-eight (49.1 per cent) of fifty-seven sexually mature female albino rats, seems to indicate that x-rays can produce a transient effect on pituitary function. Fifteen (53.5 per cent) of these twenty-eight rats accepted the male, resulting in four pregnancies and one pseudopregnancy. Is this transitory effect due to a sudden release of follicle-stimulating hormone by the pituitary as a result of an increased vascularity or increased cell permeability secondary to cellular injury following radiation? That such initial changes can be observed in tissues immediately following roentgen therapy is well known. Ewing (48) states that this initial hyperemia seems to be merely a somewhat peculiar inflammatory process with vasodilatation, exudation of serum, leukocytes, and red cell infiltration. An increased permeability of the cell membrane may result from cellular damage.

Gross studies showed the pituitaries of six of our irradiated sexually mature rats to be more erythematous in color than those of the day-two controls. In addition, the average weight of the pituitary glands of the treated rats was at least 13.9 per cent greater than that of the controls. This increase in weight of the pituitary gland following radiation is similar but slightly greater than that found in rats in normal oestrus. An increase in uterine weight is believed to be due to estrogen secretion. Though the average uterine weight was at least 12 per cent greater in the treated rats when compared to that of the controls, suggesting some increased estrogen secretion by the ovary following radiation of the pituitary, this increase in weight was not nearly as marked as the 105 per cent noted in rats during normal oestrus. This possible increased estrogen secretion was apparently not sufficient to produce an increase in running.

No EQCR response was obtained in 50.9 per cent of the fifty-seven sexually mature day-two irradiated rats. This failure may have been due to the fact that in these rats metoestrus had already become sufficiently well-established so that even though additional follicle-stimulating hormone was released following radiation of the pituitary, it was not sufficient to

the case since neither single nor serial doses of x-rays in the dosages used in this study had any effect on the development of the sexual system of sixteen immature twenty-eight day old female rats. This is presented as further evidence that x-rays cannot directly stimulate normal cellular growth.

VI. SUMMARY

Five sexually mature female Wistar albino rats were given single doses of roentgen radiation to the pituitary varying from 100 to 200 r, at 7.30 a.m. on the third day of their oestrus cycles and none appeared to be affected by treatment.

Fifty-seven sexually mature female Wistar albino rats were given single or multiple doses of roentgen radiation to the pituitary varying from 5 to 300 r, at 7.30 a.m. on the second day of their oestrus cycles.

Twenty-eight additional rats were used as controls.

Physiological signs of heat, ear quivering and copulation response (EQCR), were observed in twenty-eight (49.1 per cent) of the fifty-seven rats from one to two hours following treatment. These signs of heat lasted for six to eight hours. Fifteen of these twenty-eight rats accepted the male. Four pregnancies and one pseudopregnancy resulted. The running activity was recorded for 20 to 30 cycles after treatment. No change was noted in the running activity or periodicity of the oestrus cycle of the treated rats.

Gross anatomical studies and weight determinations were made of the pituitaries, ovaries, and uteri of six of the day-two treated rats who showed an EQCR, as well as of the seven day-two controls. The pituitary weights of the treated rats appeared to be significantly greater than those of the controls. The ovarian weights were similar in the two groups. The uterine weights of the treated rats were slightly greater than those of the controls, but this increase in uterine weight was not nearly as definite as that noted in rats during the normal oestrus.

Sixteen immature female Wistar albino rats were given either single or five successive daily roentgen treatments to the pituitary with doses varying from 5 to 100 r per treatment.

Six additional rats were used as controls.

Eight of the immature treated rats were followed until vaginal opening occurred at ages ranging from fifty-three to sixty-five days. This is within the normal limits of age for the onset of sexual maturity.

Eight additional immature treated rats and four controls were sacrificed twelve days after radiation of the pituitary, and pituitary, ovarian, and uterine weight determinations made. Grossly the appearance and weight of the organs from the treated immature rats were in no way different from their controls.

VII. CONCLUSIONS

As a result of these studies the following conclusions were reached:

1. Low dosage radiation of the pituitary can produce a transient effect on the normal pituitary function of the rat. Though the immediate physiological effect is in the nature of a stimulus, such an effect is only transient and not prolonged.
2. Radiation of the pituitary in small doses has no effect on the normal development of the sexual system of immature twenty-eight day old female rats.
3. There is no evidence that x-rays can stimulate living cells, if by stimulation is meant a continued acceleration of their normal growth or function.
4. Radiation of the pituitary in small doses has no evident harmful effect in the experimental rat.

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MYXEDEMA WITH DELAYED CLOSURE OF EPIPHYSES IN SEXUALLY MATURE WOMEN

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IT IS generally believed that closure of the epiphyses is caused by the sex hormones. In all insufficiencies of the gonads, whether primary, or secondary to insufficiency of the anterior lobe of the hypophysis, a delay in synostosis is observed. This anomaly of bone age, however, is also to be seen in thyroid deficiency. It is present not only in the myxedema of infants and adolescents but also in the myxedema of mature women who have menstruated regularly for a long time. Thus Mussio Fournier and Proto (1) have published the case of a myxedematous woman 32 years of age, untreated with desiccated thyroid, in whom menstruation had occurred with normal rhythm since the age of 22 years. In this patient some epiphyseal cartilages still existed.

Besides the case first mentioned we now present four cases of myxedematous women in whom similar conditions were observed.

CASE HISTORIES

No. 9156: P. S. was a woman 32 years of age. She was first seen by us because of a clinical picture of myxedema (Fig. 1). As an infant, teething, walking and speech followed their normal course. She was sensitive to cold from childhood and was habitually constipated. The menarche occurred at 20 years of age and was followed by regular menstruation. The symptom that brought her to our service was edema of the face and limbs which had existed for one year.

Physical examination: The patient was short, (height, 143.0 cms.) and had typically myxedematous facies, dry skin and scalp hair, scant pubic hair and no axillary hair. She was mentally deficient. Her basal metabolism was minus 28 per cent and blood cholesterol was 590 mg. per 100 cc. Roentgen ray examination of the thorax showed a dilated heart with very feeble contractions. The electrocardiogram showed low voltage. The epiphyseal lines of the upper end of the left femur were closed; the epiphyseal cartilage of the upper end of the right femur was still clearly visible. The epiphyseal lines of both iliac crests and ischia were open (Fig. 3).

A remarkable improvement occurred during treatment with desiccated thyroid, which the patient took for the first time (Fig. 2). The thyroid insufficiency apparently began after infancy, since teething, speech and walking followed a normal course.

No. 9147: M. R. was a single woman, 43 years of age (Fig. 5). The sole noteworthy fact in the family history is that her parents were tall. Regarding her personal history, the only thing known is that growth was slow and that she went to school for a year without learning anything. From childhood on, she was always listless and very slow at

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FIG. 1. P.S. before treatment.

FIG. 2. P.S. after treatment with desiccated thyroid.

FIG. 3. Open epiphyseal lines of right iliac crest (P.S. *see* Fig. 1)

FIG. 4. Synostosis of the iliac crest has not yet occurred (M.R. *see* Fig. 5)

her work. The menarche occurred at 13 years of age. Menstruation was regular, lasting from seven to eight days. She had always been constipated, sometimes moving her bowels only once a week. She had always suffered from the cold and perspired very little. Throughout life thirst had been slight and urine output low. She said that her hair was constantly falling out and she was always drowsy. Her appetite had always been poor. She consulted us because of lumbar pains of several months duration.

Physical examination: The patient presented a typical case of myxedema, with a puffy face, moderately enlarged tongue, slowness of speech, dry skin, somewhat scant, coarse, dry scalp hair, scanty pubic hair and no axillary hair. There was a small umbilical

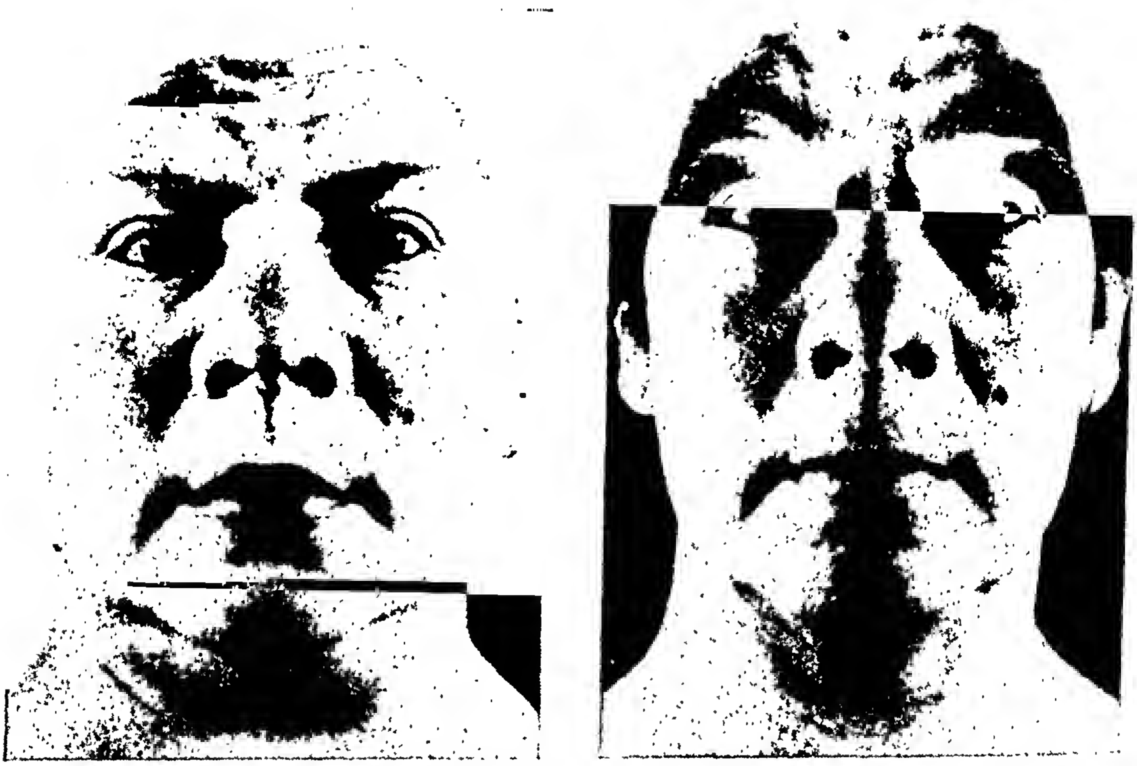

FIG. 5. M.R. before treatment.

FIG. 6. M.R. after treatment with desiccated thyroid.

hernia. Her height was only 141.0 cm. and her weight was 59.4 Kg. Her mental age was about that of a 7-year-old child. Her basal metabolism was minus 19 per cent and the blood cholesterol was 624 mg. per 100 cc. The electroencephalogram and electrocardiogram showed low voltage. Roentgenographs showed an enlarged heart and unclosed epiphyseal lines of the iliac crest (Fig. 4).

Desiccated thyroid was prescribed in a dose of 0.1 Gm. ($1\frac{1}{2}$ grs.) daily. During this treatment the puffiness of the face, the constipation, the lumbar pains, the sensitivity to cold, the dryness of the skin and hair, the drowsiness and listlessness disappeared. The electrocardiogram and electroencephalogram no longer showed low voltage. The blood cholesterol dropped to 198 mg. per 100 cc. A photograph taken six months after starting treatment is shown in Figure 6.

Here we have a case of myxedema which apparently started in childhood and was un-

treated until the age of 42. The early onset is to be deduced from her delayed mental processes, (equivalent to those of a 7-year-old girl) and from her small stature, compared with her tall parents.

No. 3310: This woman was 21 years of age. Her physical and mental development were normal until the age of 8, when growth stopped. At the age of 10 a goiter appeared and was removed by thyroidectomy. After the operation she developed puffiness of the face, listlessness, constipation, lack of perspiration, sensitivity to cold, and impairment of memory and hearing. In spite of lack of treatment with desiccated thyroid, secondary sexual characteristics appeared at the age of 15 or 16 years. The menarche appeared at the age of 19.

Physical examination: Examined for the first time at our Institute at the age of 21 years and 11 months (December 22, 1938) she presented typical myxedematous facies, dry skin, coarse dry hair, absence of axillary hair, and scant pubic hair. Her mental age was that of a girl of 13. Her height was only 132.0 cm. Her basal metabolism was minus 23 per cent. A daily dose of 0.05 Gm. ($\frac{3}{4}$ gr.) of desiccated thyroid was prescribed. After six weeks the puffiness of the face and most of the symptoms of thyroid insufficiency had disappeared. The patient took desiccated thyroid very irregularly, omitting it at times for as long as three years. In spite of the insufficient treatment, menstruation was regular. On May 4, 1945, (nine years after the menarche) vestiges of epiphyseal lines were still visible at the distal ends of the ulna and radius. On July 23, 1947, (eleven years after the menarche) synostosis of the iliac crests was still incomplete.

No. 1338: This woman was 34 years of age. As a child, first and second dentitions and speech were delayed. She began to walk at 4 years of age and showed signs of serious mental deficiency. A colloid goiter was noticed at the age of five. From her early years desiccated thyroid was taken very irregularly. The menarche occurred at 17 years of age. From then on, menstruation continued with some intervals of amenorrhea lasting from two to three months.

Physical examination: Examined at the age of 34, her height was 153.0 cm. Breasts and pubic hair were well developed. A roentgenogram of her pelvis showed the epiphyseal lines of the iliac crests still open.

DISCUSSION

We have presented here the histories of four myxedematous women in whom there was evidently a retarded closure of certain epiphyseal lines, in spite of menstruation having begun a long time previously. The menarche had appeared twelve years before in the first patient, twenty-nine years before in the second, eleven years before in the third, and seventeen years before in the fourth patient.

These facts lead one to suppose that estrogens alone are not sufficient to lead to closure of the epiphyseal lines. It would seem that the thyroid hormone plays a part in this process. It may act directly upon the epiphyseal cartilages in co-ordination with the estrogens. On the other hand, it is also possible that the thyroid hormone, by means of direct or indirect action, may induce the adrenal cortex to secrete some hormone which influences closure of the epiphyses.

In order to explain why the delay in synostosis is greater in panhypo-

pituitarism than in ovarian insufficiency. Albright *et al.* (2) have suggested that the adrenal cortex may contribute to closure of the epiphyses.

The existence of an adrenal cortex deficiency in the course of myxedema is demonstrated by decreased 17-ketosteroid excretion, and by the return of this excretion to normal after the administration of desiccated thyroid. This fact is in accord with the hypothesis we have submitted, namely, that a deficiency of the adrenal cortex may be a factor in myxedema contributing to delay in closure of the epiphyses in spite of normal ovarian function.

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THE URINARY 17-KETOSTEROID LEVELS OF HUMAN LEUKEMIC SUBJECTS[1]

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THE dependence of lymphoid organ size and weight upon adrenocortical function has been adequately demonstrated (1 to 6). Recent evidence (7, 8, 9) indicates that functional activity of lymphoid tissue is likewise intimately related to the secretory activity of the adrenal cortex.

Because lymphatic leukemia is characterized in part by hypertrophy and hyperactivity of the lymphoid tissues, it is reasonable to inquire into the possible involvement of the adrenal cortex in the leukemic syndrome. To this end, an evaluation of adrenocortical secretory activity in relation to the leukemic syndrome has been attempted. Two of the available criteria for measuring the activity of the gland have been employed. Adrenal cholesterol concentration, thought (10) to be an indicator of the secretory activity of the cortex, has been determined in mice before and after the induction of lymphatic leukemia. It was found (11) that in mice the disease is associated with a markedly decreased level of adrenal cholesterol, indicating a disturbance in the rate of secretion by the adrenal cortex.

The data presented in the present report were obtained by determination of the urinary 17-ketosteroid excretion levels of leukemic as compared to normal human subjects. The 17-ketosteroids are commonly thought to be largely derived from adrenal cortical hormones by metabolic degradation. The quantities of these substances excreted thus affords a type of measure of adrenal cortical secretory activity.

The leukemic subjects studied were patients hospitalized[3] for various treatments of the leukemic syndrome, most frequently blood transfusion. Insofar as was possible, urine specimens were taken before any "adrenal-activating" treatment (such as irradiation or administration of arsenicals) was instituted. The diagnoses and types of leukemia are indicated in appropriate places. The control subjects were healthy laboratory personnel presumed to be nonleukemic.

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[3] The leukemic subjects were patients of Dr. Raphael Isaacs, who made the diagnoses of the various types of leukemia indicated.

A slight modification of the method of Callow, Callow and Emmens (12) was used for the 17-ketosteroid determinations. It is to be noted that the fractionation procedure included treatment with Girard's Reagent T in order to eliminate nonketonic chromagens.

RESULTS

The data derived from 39 determinations of 17-ketosteroid excretion by 8 leukemic and 6 normal subjects are presented in Table 1.

TABLE 1. URINARY 17-KETOSTEROID EXCRETION BY NORMAL AND LEUKEMIC SUBJECTS

Subject	Age	Diagnosis	Leucocytes		17-Ketosteroid Excretion	
			Total	Blasts	Individual	Average
	(yrs.)		×1000	per cent	mg./24 hrs.	mg./24 hrs.
♂ N.I.	25	normal			6.4; 9.3; 8.9	8.2
♂ H.S.	32	normal			7.6; 4.9; 6.2	6.2
♂ L.L.	37	normal			10.2; 8.4; 7.0; 7.5	8.5
♂ H.A.	67	chronic lymphatic leukemia	70-180	0-1	0.0; 2.5; 2.8; 1.3; 0.8	1.5
♂ P.B.	7	chronic lymphatic leukemia	40-52		2.4	2.4
♂ S.La.	49	acute aleukemic leukemia	0.2-3.9	50-86	2.4	2.4
♂ L.H.	51	acute myelogenous leukemia	5.7-13.8	24-60	10.6; 6.3; 5.0; 6.1; 5.0	6.6
♂ A.F.	28	acute undifferentiated leukemia	21-70	70-95	11.1; 3.9; 9.3; 9.8; 7.6; 7.1; 8.1.	8.1
♀ E.H.	36	normal			4.1	4.1
♀ E.W.	34	normal			6.0; 4.8	5.4
♀ B.S.	30	normal			5.6	5.6
♀ W.	74	chronic lymphatic leukemia			0.9	0.9
♀ F.	50	acute monocytic leukemia	18-120	7-79	2.1; 3.2; 1.7; 3.5	2.6
♀ H.	66	chronic myelogenous leukemia	51-155	2	5.8	5.8

Inspection of the data shows that all three of the lymphatic leukemic subjects excreted markedly reduced amounts of 17-ketosteroids as compared to nonleukemic individuals of the same sex. On the other hand, those patients having leukemia other than the lymphatic variety (one acute myelogenous; one chronic myelogenous; one acute undifferentiated) excreted quantities of 17-ketosteroids of the same magnitude as did normal subjects of the same sex. Two exceptions were the man with acute aleukemic leukemia and the woman with acute monocytic leukemia, both of whom excreted small quantities of 17-ketosteroids. However, it does not seem unlikely that each of these two types may be more closely related, physiologically, to lymphatic than to myelogenous leukemia.

It is to be noted that the 17-ketosteroid excretion levels were not well correlated with clinical conditions. Thus, ♂ A.F., suffering from acute undifferentiated leukemia and almost moribund at the time the urine collections were made, excreted normal amounts of 17-ketosteroids. On the other hand, ♂ H.A., although he had only a mild case of chronic lymphatic

leukemia, excreted greatly reduced quantities of 17-ketosteroids. The lack of correlation between clinical condition and 17-ketosteroid excretion level suggests that debilitation was not the crucial factor here.

The results are not vitiated by the fact that most of the leukemic subjects were in the older age range. It may be noted, for example, that ♂ L H., aged 51, excreted normal quantities of 17-ketosteroids while the urine of ♂ S. La., aged 49, showed a markedly reduced 17-ketosteroid titer. Likewise, ♀ H., aged 66, excreted normal amounts, whereas ♀ F, aged 50, excreted but little 17-ketosteroid.

It thus would appear that only lymphatic leukemia, even in relatively mild chronic cases, is associated with decreased urinary 17-ketosteroid excretion. This finding correlates very well with the report of Dobriner *et al.* (13) that the qualitative distribution pattern of urinary 17-ketosteroids is deranged in lymphatic but not in myelogenous leukemia.

The present results are not interpreted as pointing to the adrenal cortex as a causative agent in lymphatic leukemia. However, when considered together with the results obtained from leukemic mice (11) as well as with the results of other laboratories (13), they suggest that the possible participation of the adrenal cortex in the leukemic syndrome merits further investigation.

SUMMARY

The urinary 17-ketosteroid level of 8 human leukemic subjects has been studied. Markedly subnormal quantities were excreted by 5 of the subjects in whom the diagnoses were chronic lymphatic leukemia (3 cases), acute aleukemic leukemia and acute monocytic leukemia. The 17-ketosteroid excretion was not depressed in three additional patients with leukemia, one with the acute and one with the chronic myelogenous variety and one with the acute undifferentiated type in the terminal stages. These findings indicate a need for further study of the possible participation of the adrenal cortex in the lymphatic leukemia syndrome.

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PROGRAM OF THE 1948 LAURENTIAN HORMONE CONFERENCE

Forest Hills Hotel, Franconia, New Hampshire
September 13-18

Because of limited accommodations, attendance is by invitation. Correspondence should be addressed to: Dr. Gregory Pincus, Chairman, Committee on Arrangements, 222 Maple Avenue, Shrewsbury, Massachusetts.

I. STEROID HORMONE METABOLISM "IN VIVO" AND "IN VITRO"

Some aspects of Progesterone Metabolism
DR. G. F. MARRIAN, *University of Edinburgh*
Monday Evening, September 13

Recent Developments in Our Knowledge of Estrogen Metabolism
DR. R. D. H. HEARD and MRS. J. C. SAFFRAN, *McGill University*
Tuesday Morning, September 14

The Metabolism of Androgens by Tissues
DR. LEO T. SAMUELS, *University of Utah*
Tuesday Morning, September 14

The Metabolism of Estrogens with Particular Emphasis on Clinical Aspects of Physiology and Function of Ovarian Hormones
DR. ALBERT SEGALOFF, *Alton Ochsner Medical Foundation*
Tuesday Evening, September 14

II. THE ROLE OF HORMONES IN TISSUE AND BODY METABOLISM

The Antihormone Problem in Endocrine Therapy
DR. JAMES H. LEATHEM, *Rutgers University*
Wednesday Morning, September 15

Integration of the Metabolic Effects of Adrenal Cortical, Thyroid and Growth Hormones
DR. ABRAHAM WHITE, *University of California at Los Angeles*
Wednesday Morning, September 15

The Alterations in Metabolism Incident to Administration of Insulin, Adrenalin and Thyroid Substances, Studied with the Aid of Isotopes

Dr. DeWitt Stetten, Jr., *Harvard University Medical School*

Wednesday Evening, September 15

The Pancreas as the Guardian of the Liver

Dr. Charles H. Best, *University of Toronto*

Thursday Morning September 16

Metabolic Changes in Man Following Adrenal and Pituitary Hormone Therapy

Dr. George W. Thorn, *Harvard University Medical School*

Thursday Morning, September 16

III. NEUROHUMORAL-HYPOTHALAMIC RELATIONSHIPS

Adrenal Function in Mental Disease

Dr. Gregory Pincus, Dr. Hudson Hoagland, Dr. Harry Freeman and Mr. Fred Elmadjian, *Worcester Foundation for Experimental Biology*

Thursday Evening, September 16

Manifestations of Altered Autonomic and Humoral Functions in Psychoneuroses

Dr. Robert A. Cleghorn, and Dr. B. F. Graham, *McGill University*

Friday Morning, September 17

Effects of Hypothalamic Lesions on Water and Energy Metabolism in the Rat

Dr. James A. F. Stevenson, *Yale University*

Friday Morning, September 17

IV. THYROID PHYSIOLOGY AND FUNCTION

Physiologic Reactions of the Thyroid Stimulating Hormone

Dr. Rulon W. Rawson and Dr. William L. Money, *Massachusetts General Hospital*

Friday Evening, September 17

The Metabolism of Iodine as Disclosed by the Use of Radioiodine (I131)

Dr. F. R. Keating, Jr., and Dr. Alexander Albert, *The Mayo Clinic*

Saturday Morning, September 18

Radioiodine as a Diagnostic and Therapeutic Tool in Clinical Medicine

Dr. S. M. Seidlin, *The Montefiore Hospital*

Saturday Morning, September 18

Abstracts of
CURRENT ENDOCRINE LITERATURE

ADRENALS

CLARKE, A. P. W.; CLEGHORN, R. A.; FERGUSON, J. K. W., and FOWLER, J. L. A.: Factors concerned in the circulatory failure of adrenal insufficiency, *J. Clin. Investigation* 26: 359–363, 1947.

In an effort to determine the factors concerned in the circulatory failure of adrenal insufficiency, changes in blood and interstitial fluid volumes have been observed in 12 experiments upon 8 adrenalectomized dogs in crisis and under adequate treatment with cortical extracts. In conjunction with treatment, attempt has also been made to appraise the influence of desoxycorticosterone acetate and a low salt intake. The plasma volumes were measured by the use of the blue dye T 1824 according to a modification of the procedure of Gregerson and Stewart. Total blood volumes were calculated from the plasma volume. The volume of interstitial cell fluid was determined by the sulfocyanate method. In crisis, plasma volumes were about 30 per cent, total blood volumes 17 per cent and interstitial fluid volumes five per cent to ten per cent below the normal. Three different types of regime for controlling the adrenal insufficiency were used: I. cortin, 1 cc. twice daily plus a diet containing two per cent sodium chloride; II. cortical extract, 4 cc. twice daily, plus one per cent sodium chloride; and III. desoxycorticosterone acetate, 1.25 mg. daily, plus a diet containing one per cent sodium chloride. In each instance, determinations were made during periods of adequate control and in crisis following withdrawal of treatment. Changes in the interstitial fluid varied considerably and were most marked in those animals to which desoxycorticosterone acetate was given. Groups I and II behaved similarly throughout periods of control and crisis. Plasma volume was distinctly higher in group III, and, while values for interstitial fluid varied considerably in all groups and from animal to animal, such changes were most marked in those animals that received desoxycorticosterone acetate. Following the withdrawal of cortin from those animals that had simultaneously received extra salt during the control period. the intake of water and the output of urine were both markedly increased, suggesting interference with the kidney's capacity for excreting a urine containing a high concentration of salt. Observations not reported in the present communication showed "that there is a considerable loss of protein, especially albumin, from the circulating plasma during the development of adrenal insufficiency." On the basis of the data presented, several conclusions have been drawn. Factors concerned in the decreased plasma volume observed in the crisis of adrenal insufficiency include a loss of total

extra-cellular fluid due to a loss of sodium and a retention of potassium and a reduction in plasma proteins with a resulting loss of plasma to the extravascular spaces. The reduction in plasma volume alone does not account for the circulatory failure found in crisis. Cardiac failure, "together with decreased capillary tone" may be responsible "for the severe circulatory failure associated with the apparently moderate decrease in blood volume." Renal excretion accounts for the major portion of the loss of interstitial fluid, but there is also a significant shift to intracellular spaces.—*T. H. McG.*

DOUGHERTY, T. F., and WHITE, A.: An evaluation of alterations produced in lymphoid tissue by pituitary-adrenal cortical secretion, *J. Lab. & Clin. Med.* **32**: 584–605, 1947.

This article represents a review and critical evaluation of the rather extensive work of the authors and of others on the pituitary-adrenocortical regulation of the activity of lymphoid tissue. The known influences of the hormones of these two glands upon lymphoid tissue may be summarized under several headings: 1) *Lymphoid tissue weight:* Injections of adrenotropic hormone of the pituitary caused a significant decrease in the weight of the lymphoid tissue of rats and mice, with the most marked decrease occurring in the thymus gland. The spleen enlarged, due to edema and to the accumulation of degenerating lymphocytes but its Malpighian corpuscles were markedly decreased in size. Adrenalectomy reversed these processes with a marked hyperplasia of all the lymphoid organs. Desoxycorticosterone acetate was without influence on the lymphoid tissues in either the normal or adrenalectomized animal. 2) *Adrenal size and cholesterol content:* A single injection of adrenotropic hormone caused a sharp drop in adrenal cholesterol. Repeated daily injections were associated with an increase in weight and a rise above normal in the values for cholesterol. 3) *Circulating lymphocytes:* Single and repeated insections of adrenotropin produced a lymphopenia, which was maintained throughout the experimental period (observations have been made through 15 days of daily treatment). Adrenalectomized animals with and without desoxycorticosterone acetate therapy showed a significant leucocytosis and lymphocytosis; which was not due to hemoconcentration. The lymphopenic reaction began within one hour and reached its maximum within nine hours following a single injection of the pituitary hormone. In the absence of the adrenal, pituitary adrenotropin exerted no effect upon the circulating lymphocytes. Injections of watery extracts of the adrenal, corticosterone and 11-dehydro-17-hydroxy-corticosterone decreased the lymphocytes of the blood in normal and adrenalectomized animals. Desoxycorticosterone acetate had no such effect. A wide variety of physical and chemical stresses produced lymphopenia in normal rats and mice, but failed to do so in adrenalectomized or hypophysectomized animals. 4) *Circulating polymorphonuclear leucocytes:* No specific influence upon the polymorphonuclear elements of the blood could be observed following the administration of pituitary adrenotropic hormone or steroid hormones of the adrenal cortex. Likewise, hypophysectomy and adrenalectomy were without definitive action. 5) *Circulating erythrocytes:* Both adrenotropin and adrenal cortical hormones caused an increase in hemoglobin and the number of circulating red cells. Conversely, adrenalectomy tended to decrease them. 6) *Histologic changes in lymphoid tissue:* A single injection of adrenotropin or adrenal cortical hormones was followed by dissolution of lymphocytes, reaching its acme within from one to six hours. Concomitantly, there was an increase in the amount and alteration in the structure of the histiocytes. Following the initial dissolution of lymphocytes, cellular debris was removed by macrophages. Within six to nine hours most of the nuclear fragments had been phagocytized, lymphocytic dissolution had ceased, edema had

subsided, and a normal lymphoid structure began to reappear. Within 24 hours there was relatively complete recovery in all lymphoid structures except the thymus. In adrenalectomized animals, neither adrenotropin nor desoxycorticosterone acetate (doses ranging from 0.025 mg. to 2.5 mg.) was capable of eliciting the above responses, but the other adrenal cortical preparations mentioned above were equally active in normal and adrenalectomized subjects. Nonspecific stress stimuli produced changes in the lymphoid tissues of the intact animal, but were without effect in those that had been adrenalectomized. 7) *The release of blood globulin and antibody from lymphocytes:* Adrenotropin and adrenal cortical hormones produced a statistically significant increase in the level of total serum proteins in intact animals. The latter were equally effective in the adrenalectomized animal, but the former failed completely to influence the decrease in the protein of the blood that ordinarily follows adrenalectomy. The increase in protein appeared almost wholly in the beta and gamma globulin fractions thus suggesting the relationship of the phenomenon to factors concerned in antibody formation. When the same hormones were used under equivalent conditions in immunized animals, an increase in the production of antibodies was routinely observed. These alterations in serum protein and in antibody production were always maximal when lymphopenia was highest and lymphocytic dissolution greatest. Extension of these experiments made it clear that the anamnestic response is probably mediated through pituitary stimulation resulting in a release of antibodies from the lymphocytes. The authors conclude that "the role of the adrenal cortex in controlling the rate of release of lymphocyte constituents correlates the functions of the adrenal and of the lymphocyte in resistance. The participation of the adrenal cortex is dependent upon its stimulation by pituitary adrenotropic hormone."—*T. H. McG.*

MAYCOCK, R. L., and ROSE E.: Insensitivity to epinephrine in a patient with a functioning tumor of the adrenal medulla, *Am. J. M. Sc.* **213**: 324, 1947.

The case history is reported of a 42-year-old male who experienced one to 16 attacks daily for two and one-half years before admission for paroxysmal hypertension and associated symptoms. The physical examination was essentially normal, and the blood pressure was 130/80 mm. of mercury when the patient was free from attacks. Attacks were often brought on by physical exercise or changes in position. Detailed clinical description of the attacks is given. A tumor weighing 42.8 Gm. and containing 76.5 mg. of free epinephrine was successfully removed from the region of the right adrenal. Histologically the morphology was consistent with the usual pheochromocytoma or adrenal paraganglioma. Prior to operation, studies were made to discover what stimuli were effective in producing an attack. Typical paroxysms were induced by exercise and postural changes, such as having the patient bend and twist his waist or press his knees into his abdomen, and by the subcutaneous injection of 10 mg. of acetyl-beta-methyl choline. Emotion, manual massage of the adrenal area, injections of sterile water, and inhalation of a perle of amyl nitrite were ineffectual in producing an attack. The most striking demonstration was the insensitivity of the patient to epinephrine: 2.5 cc. of a potent 1:1000 solution were needed to produce a blood pressure rise to 220/114 and the symptoms that usually occurred with a mild attack. After the tumor had been removed, injection of 0.25 cc. of 1:1000 epinephrine solution subcutaneously induced a rise in blood pressure to 172/98 with all of the previous symptoms of a typical attack. This time, however, he complained of nervousness which he had not experienced before even during his worst episodes. Twenty-three minutes after the onset of a severe attack, the

serum potassium level was depressed to 2.9 m. Eq./100 cc. (normal range 3.8 to 4.3); when no attack was present, the level was elevated to 5.0 m. Eq./100 cc.; at operation the potassium content of the rectus muscle was found to be 172.0 m. Eq./Kg. of wet muscle (normal 82 m. Eq./Kg.). During an attack 0.45 mg. of conjugated epinephrine was excreted per 100 cc. of urine. The amount of choline esterase in the blood was normal during the interval between attacks. Additional data are given, including the values for other chemical constituents of the blood, and the electrocardiograms and ballistocardiograms before, during, and after attacks. The authors conclude that the patient was hyposensitive to epinephrine action while the medullary tumor was present and was normally sensitive to this drug when the tumor had been extirpated. They suggested that the demonstration of such insensitivity may serve as a useful and harmless test for the diagnosis of such tumors.—*E.C.R., Jr.*

SELYE, FRANCES L.: Biochemical changes in hypertension, *Canad. M.A.J.* **57**: 325, 1947.

In this study an attempt is made to find criteria for an adrenocortical etiology in certain cases of "essential" hypertension. On the basis of animal experiments of H. Selye, and of clinical studies in Cushing's syndrome (Kepler), a high Na/Cl ratio in the serum is considered suggestive of hypersecretion of "mineralo-corticoids." Several of these patients also had high "glycocorticoid" with low 17-ketosteroid excretion. Ammonium chloride therapy was given under adequately controlled conditions, including control periods of placebo medication. This therapy was suggested by the fact that in animal experiments vascular changes from desoxycorticosterone plus salt administration failed to occur when ammonium chloride was given simultaneously. The results of this therapy were encouraging.—*K.E.P.*

GONADS

GUTERMAN, H. S.: Influence of arginine on oligospermia, *Proc. Soc. Exp. Biol. & Med.* **65**: 176–178, 1947.

Observations were made on 23 patients with oligospermia. Eighteen received from 1.8 Gm. to 2.7 Gm. of arginine and minimal amounts of lysine, pyridoxine and tryptophane in tablet form daily for eight months and five patients received no specific therapy. All of the men had histories of adequate protein intake. No improvement in semen quality which could be attributed to treatment was produced and it was concluded that amino acid therapy for oligospermia should be reserved for patients who exhibit or give the history of inadequate protein intake.—*F.N.A.*

HARTMAN, C. G., and CORNER, G. W.: Removal of the corpus luteum and of the ovaries of the Rhesus monkey during pregnancy: observations and cautions, *Anat. Rec.* **98**: 539, 1947.

In a series of experiments on the physiology of reproduction in the monkey it was found that the corpus luteum is not essential to the continuation of pregnancy after the twenty-fifth day. Complete surgical removal of the ovaries in the monkey is attended with great difficulty and the authors hold that in primates, total removal must be proven by the subsequent absence of cyclic phenomena, by microscopic evidence that the excised ovaries were removed intact and by a thorough search for residual ovarian tissue.—*B.L.B.*

KIMMEL, G. C. and WING, R.: Sexual precocity and accelerated growth in a child with a follicular cyst of the ovary, *J. Ped.* 30: 686, 1947.

The authors report the case history of a 25-month-old girl who showed all the changes observed in granulosa-cell tumor of the ovary, including a greatly increased urinary estrogen level. At operation, the right ovary was found to consist chiefly of a cyst 4 by 3 cm. in diameter which had destroyed most of the ovarian tissue. Careful examination of the cyst revealed no granulosa-cell tumor and it was classified as a follicular cyst of the ovary. The pathologist suggested that sufficient estrogen to cause the changes noted in this patient might have been produced by the granulosa cells which lined the cyst. During the following four years there has been no recurrence of vaginal bleeding; the breasts, the nipples and the labia have regressed to their normal size; and the estrogen content of the urine has returned to and remained at a normal level. The patient has remained above average height and weight during these subsequent years. No abnormal values for urinary ketosteroid excretion were found at any time.—*E.C.R., Jr.*

LEONARD, S. L.; PEARLMAN, P. L., and KURZROCK, R.: Relation between time of fertilization and follicle cell dispersal in rat ova, *Proc. Soc. Exp. Biol. & Med.* 66: 517–518, 1947.

Following the discovery that the enzyme hyaluronidase from sperm disperses the follicle cells of recently ovulated mammalian ova, a concept has arisen that the action of hyaluronidase facilitates fertilization by denuding the ova prior to or simultaneously with sperm entry. The observations reported in this paper indicate no mass removal of the follicle cells prior to fertilization and that sperm penetration precedes the gross denudation of the ovum in the rat. Female rats were bred and the ova examined microscopically 12 to 26 hours later. In another experiment 0.2 cc. of hyaluronidase from bull or rat testes, in concentrations of 30 to 60 turbidity reducing units per cc., was introduced into each horn of the uterus of a rat in heat and the horns ligated near the cervix to prevent leakage. It was found that fertilization of the rat ovum occurred before mass displacement of the surrounding follicle cells; denudation of the ova occurred subsequently. Hyaluronidase introduced into the uterus did not pass into the tubes and denude the ova and it appeared that only the enzyme associated with the sperm which reach the oviduct, disperses the follicle cells.—*F.N.A.*

REIFENSTEIN, E. C., JR., and ALBRIGHT, F.: The metabolic effects of steroid hormones in osteoporosis, *J. Clin. Investigation* 26: 24–56, 1947.

The authors define osteoporosis as "that form of undermineralization of bone in which the primary defect is a hypofunction of the osteoblasts in laying down bone matrix." Sharply distinguished are osteomalacia, where there is a failure in mineralization; and hyperparathyroidism, where there is an increased destruction of bone. Osteoporosis in the sense used occurs in malnutrition (protein), Selye's adaptation syndrome, idiopathic osteoporosis, acromegaly, Cushing's syndrome, disuse atrophy, old age, and, commonest of all, the postmenopausal state. Twelve patients with osteoporosis are described in detail. In eleven the results of therapy with estrogens, androgens and progesterone are described. These subjects represent the last four categories mentioned above. Estrogens caused a decrease in calcium and phosphorus excretion in the subjects of all four groups. They usually caused a decreased fecal calcium and phosphorus as well. A moderate but not well sustained decrease in urinary nitrogen and 17-ketosteroids

was observed. Effective dosages for estradiol benzoate intramuscularly ranged from 1.66 mg. every three days to 3.32 mg. daily; and, for stilboestrol orally, from 1 mg. to 15 mg. daily. The smallest of these doses was usually effective with very little if any increase in activity as increments in dosage were made. No qualitative difference could be observed between the natural and the synthetic estrogen. The effects of the hormones on calcium and phosphorus balance were demonstrable by the sixth day, reached their maximum around the thirtieth day, and persisted from one to two months following the cessation of treatment. Androgens in the form of testosterone propionate intramuscularly (25 mg. to 50 mg. daily) and methyl testosterone by mouth (40 mg. to 100 mg. daily) decreased the urinary and fecal excretion of calcium and phosphorus in osteoporosis. These effects made their appearance about the fifth or sixth day as with the estrogens, but their acme occurred somewhat later, and they disappeared more slowly after withdrawing the drug. The decrease in urinary nitrogen was marked and prolonged. Testosterone propionate and methyl testosterone were equally effective. When androgen and estrogen were combined in the postmenopausal and senile groups the effect was greater than that observed following the administration of either alone. Progesterone, intramuscularly, in doses of 10 mg., 25 mg., and 100 mg. daily, for as long as 36 days, was without appreciable effect upon the urinary and fecal excretion of calcium, phosphorus or nitrogen, whether it was used alone or in combination with one of the other hormones. Except in Cushing's syndrome, the beneficial effects of estrogens upon the bony disturbance were more marked than those of testosterone. The authors call particular attention to their regime for the management of the postmenopausal type of osteoporosis. In order to avoid troublesome bleeding and to decrease the risk of inciting a precancerous lesion into full activity, treatment is usually interrupted every four to seven weeks for one to two weeks, and often a short course (five days) of progesterone is employed during this interval. Vaginal smears are used periodically in an effort to detect any neoplastic change early.—*T.H.McG.*

TAYLOR, R. D.; CORCORAN, A. C., and PAGE, I. H.: Menopausal hypertension: A critical study, *Am. J. M. Sc.* 213: 475, 1947.

Since the concept "menopausal hypertension" appeared to have been derived merely from accumulated impressions rather than from systematic study, the authors studied the incidence of hypertension in 200 menopausal women, 179 of whom had been surgically castrated and all of whom desired relief of menopausal symptoms. The results showed that arterial hypertension is no more common in menopausal women than in the general population. The authors believe that "vasomotor instability," as exhibited by "hot flashes," perspiration, and tachycardia, is not necessarily associated with hypertension and its alleviation by estrogens need not affect arterial pressure. In general, the menopause seemed to intensify preexisting psychoneuroses. Despite severe neurotic behavior, hypertension did not develop within three or more years except in 6 cases. The authors conclude that the relationship between the menopause and hypertension is incidental, and that loss of ovarian secretion is neither a primary nor a contributory cause of arterial hypertension.—*E.C.R., Jr.*

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ADRENAL CORTICAL TUMOR ASSOCIATED WITH CUSHING'S SYNDROME

REPORT OF A CASE WITH METABOLIC STUDIES AND REMARKS ON THE PATHOGENESIS OF CUSHING'S SYNDROME

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THIS case report and the metabolic studies that accompany it are presented primarily because of their bearing on the pathologic physiology of Cushing's syndrome. The term "Cushing's syndrome" as used herein refers only to the clinical picture emphasized by Cushing. The term "Cushing's disease" will be used in a broader sense than we used it in previous publications so as to denote all cases of Cushing's syndrome in which an adrenal cortical tumor is not present.

REPORT OF CASE

A married woman aged twenty-six years first registered at the Mayo Clinic on September 3, 1943. There was nothing remarkable in her past medical history. On June 23, 1941, she consulted her physician because she was eight weeks pregnant. At that time she weighed 110 pounds (50 Kg.). The blood pressure expressed in millimeters of mercury, was 100 systolic and 60 diastolic, and the patient seemed to be a normal pregnant

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woman. Pregnancy proceeded uneventfully until December 22, 1941, when she was delivered of a premature infant who died shortly after birth. On February 21, 1942, she had one scant menstrual period. No further menstrual bleeding occurred until May, 1942, when she began to have daily spotting of blood. The following month a dilatation and curettage was performed. The endometrium was found to be "hyperplastic." After this operation, vaginal bleeding and menses ceased. In January, 1943, she had a renal

FIG. 1. Patient at time of initial examination. Note thin arms and legs, striae, protuberant abdomen and cervicodorsal hump.

colic on the right side and may have passed a stone. Investigation of the urinary tract revealed only hydronephrosis on the right.

About May, 1943, a new train of symptoms began. Hair began to appear on her upper lip, cheeks, legs and arms. The hair of the scalp began to fall out. Her face began to swell and her eyes seemed to protrude. A "bump" appeared on the back of the neck. Her friends noted that she "looked fatter" and that her "expression" was different. She became very nervous and apprehensive. One of the most distressing symptoms was muscular weakness, particularly of the legs. Walking and climbing stairs became difficult and she had to spend much of her time in bed. She also suffered from transitory attacks of

numbness of the entire right half of the body. In May, 1943, it was discovered that erythrocytosis (7,000,000 erythrocytes per cubic millimeter) was present. Because of this she was given medicine to "thin the blood," and in addition she was "treated with hormones." By August, 1943, she presented most of the symptoms of Cushing's syndrome, and she was referred to the Clinic with the suggestion that she might have a tumor of the adrenal cortex.

At examination, September 3, 1943, the patient's height was 66$\frac{3}{4}$ inches (169.5 cm.) and her weight was 125 pounds (56.7 Kg.). The patient was pleasant and co-operative but obviously alarmed about her condition. She had thin arms and legs, a protruding abdomen and a moderate kyphosis (Fig. 1). The face was full, round and highly colored. Hair was present on the upper lip, the cheeks, arms and legs. At the temples, the hair line was receding and she seemed to be losing some hair at the crown of the head. Although the eyes were slightly prominent, the upper lids drooped. The corners of the mouth likewise drooped. Purplish striations were present on the buttocks. There was no acne. Large and deeply pigmented areolae made the breasts conspicuous. The labia majora were slightly edematous. The clitoris was normal in size.

On pelvic examination it was suspected that a retro-uterine mass might be present. Contrary to the uterine atrophy which is often found in cases of Cushing's syndrome, in this case the uterus seemed to be normal in size. Although the circumference of the arms and legs was reduced, the panniculus of fat was well preserved. The muscles felt soft and flabby. Muscular weakness was easily demonstrated in all four extremities. The extensor groups were weaker than the flexors, and the lower extremities seemed to be weaker than the upper. Tendon reflexes were all present. The blood pressure was 204 systolic and 110 diastolic. In the ocular fundi the arterioles were slightly narrowed and, in some, localized spastic constrictions were noted.

An excretory urogram showed slight caliectasis on the right side. Above the left kidney, which seemed to be displaced downward, there was suggestive evidence of a soft-tissue mass. These urologic findings suggested, but did not prove, the presence of an adrenal tumor on the left. Roentgenograms of the head, thorax and extremities did not reveal any abnormality. Evidence of a slight degree of osteoporosis was noted in the roentgenograms of the pelvis. The results of additional laboratory studies conducted at this and subsequent visits are recorded in Table 1.

After the initial clinical examinations were concluded, a diagnosis was made of Cushing's syndrome associated with tumor of the adrenal cortex.

In order to permit study of the metabolism of electrolytes and the urinary excretion of steroids, the patient was admitted to the metabolic unit of the Clinic. The observations that were made there are presented elsewhere in this report.

On October 15, 1943, after completion of the metabolic studies, both adrenal glands were explored surgically by way of bilateral posterolumbar incisions. Because the patient complained of pain in the right flank, the right adrenal gland was explored first. It appeared to be normal and was not disturbed. On the left side a large, fairly well-encapsulated adrenal tumor was encountered and enucleated without difficulty. After removal, the tumor measured 10 by 15 by 8 cm., weighed 410 Gm., was yellowish in color and was surrounded by a plexus of veins. The capsule was ruptured at several points.

In order to prevent the occurrence of acute adrenal insufficiency postoperatively, the patient was treated before and immediately after the operation with desoxycorticosterone acetate and adrenal cortical extract. Details of the treatment employed are recorded in Table 2.

Convalescence proceeded uneventfully, and three weeks after the operation the metabolic studies which had been instituted before the operation were continued. The patient remained under observation until December 3, 1943. After the operation there was a gradual decrease in the blood pressure, and prior to her dismissal the daily readings were normal, ranging from 120 to 130 systolic and from 60 to 70 diastolic. She was feeling very well. Her strength was returning. The arms and legs were beginning to take on their normal shape, and it was roughly estimated that the muscle tissue palpable in the calves had increased 30 to 40 per cent. Venipuncture no longer induced ecchymosis. Her facial contours were less round. New hair was appearing in the scalp and the excess hair on the thighs was disappearing. The skin lost its sandpaper appearance and texture and it appeared to be entirely normal, except for the purplish striae, which remained unchanged.

TABLE 1. LABORATORY FINDINGS

	September 1943 (before operation)	October-November 1943 (after operation)	January, 1945 (after recurrence of tumor)
Urine			
Specific gravity	1.026	1.017	1.017
Reaction	Alkaline	Acid	Alkaline
Albumin, grade*	2	1	2
Sugar, grade*	1	0	1
Microscopic:			
Pyuria, grade*	1	3	1
Other findings	Gram-positive cocci		Occasional erythrocyte
Blood			
Hemoglobin, gm.†	17.8	12.0	11.05
Erythrocytes‡	4,950,000	3,620,000	3,020,000
Leukocytes‡	10,200	8,000	11,700
Comment	§		
Urea, mg.†	23	23	38–106
Sugar, mg.†	83		
Hematocrit‖	56.9	35.7	46
Sodium, mEq.¶	146.0	140.0	149.0
Potassium, mEq.¶	4.4	4.7	2.2
Carbon dioxide, mEq.¶	34.2	28.4	39.2
Chloride, mEq.¶	96.0	104.0	92.0
Calcium, mg.**	9.84	9.7	
Phosphorus, mg.**	1.76	4.81	
Phosphatase, alkaline, Bodansky units**	4.0		
Protein, Gm.**	7.0		

TABLE 1. (*continued*)

	September 1943 (before operation)		October-November 1943 (after operation	January 1945 (after recurrence of tumor)
Miscellaneous	Sugar, mg.†			
Glucose tolerance	Blood	Urine		
Fasting	77	0		
½ hour	112	0		
2 hours	154	0		
3 hours	104	0		
Blood volume				
Plasma, c.c.	1,971 (37 cc. per Kg.)			
Whole blood, c.c.	4,584 (86 cc. per Kg.)			
Gonadotropic hormone (prolan), rat units per liter (Frank technic)	Less than 5			
Urinary 17-ketosteroids, mg. per 24 hours	136		2.7	110 (60% beta fraction)†† 314‡‡
Urinary estrogens, rat units per 24 hours	9,400		43	5,000+
Sedimentation rate, mm. in 1 hour	10			
Basal metabolic rate, per cent	0		+15	

* Graded on the basis of 1 to 4, in which 1 represents the mildest and 4 the most severe condition.

† Per 100 c.c. of blood.

‡ Per cubic millimeter of blood.

§ Increased regeneration, piling of erythrocytes and leukocytes; suggest polycythemia vera.

‖ Per cent of cells.

¶ Per liter of plasma.

** Per 100 c.c. of serum.

†† June, 1944.

‡‡ January, 1945.

Her condition in the next few months can be described best by excerpts from her letters. On February 16, 1944 she wrote: "I'm getting along fine. Haven't felt bad at all. I feel much stronger and my appetite has come back. Also, my hair has grown so that

today I was able to get a 'permanent.' I went to see my doctor, and he said I was beginning to look like someone he used to know. That made me feel good." On March 16, 1944 she said: "I am still feeling better, but since I last wrote I have had trouble with my monthly periods. I had a normal period on the tenth of December and one on the seventh of January, but I completely skipped February and, so far, March. My hair is much thicker now and I have regained most of my strength. The above is the only thing that is not returning to normality." On April 19, 1944 she stated: "My periods have not recurred and for the past week I have had pains in my back similar to the ones I used to have. However, as a whole, I am feeling better." On May 29, 1944 she wrote: "Everything seems to be going wrong again, so I am coming back to the clinic for a checkup."

TABLE 2. TREATMENT BEFORE AND AFTER REMOVAL OF ADRENAL CORTICAL TUMOR

Year, 1943	Desoxycorticosterone acetate, mg.	Cortical extract (Kendall), c.c.	Physiologic saline solution, c.c.
Preoperative			
October 14	10		
15	10	50	1,000
Postoperative			
15		50 45 30	1,000 1,000 1,000 U.R.*
16		30 30 30 30	1,000 1,000
17		10 10	1,000

* "U.R. solution" contains, in one liter, 2.8 Gm. of sodium chloride, 0.9 Gm. of potassium chloride and 30 Gm. of glucose.

When she returned on June 4, 1944, it was immediately apparent that she probably had a recurrence of the tumor. The face again was full and round; there was very extensive acne of the face, chest and back. The hirsutism had returned. The arms and legs were again thin, and there were marked cutaneous striations in the vicinity of the hip joints. The blood pressure was 140 systolic and 118 diastolic. Various laboratory studies were again conducted with essentially the same results as those obtained prior to operation. Urinary excretion of the 17-ketosteroids was 110 mg. per 24 hours, of which 60 per cent was of the beta type. Urinary estrogens were not determined.

Previous experience with cases of recurring carcinoma of the adrenal cortex had convinced us of the futility of roentgen therapy. The patient, however, was desperate and wanted something done. Although it seemed unlikely that further surgical treatment could accomplish anything, it was decided to explore the left adrenal region once more. Through a second posterolateral incision the left kidney was brought down, and at the upper pole a large mass could be felt. Superficially, the mass seemed well encapsulated, but as the operation proceeded, it was found that the recurrent tumor was very extensive and adherent to the surrounding structures. To remove the tumor it was necessary to remove the left kidney. The operation was very difficult to perform, and at its completion it was felt that all of the tumor had not been removed. The same preoperative and postoperative treatment that had been used at the first operation was again employed, and again the patient's postoperative convalescence proceeded uneventfully. From this operation the patient received very little benefit, although the urinary excretion of 17-ketosteroids was found on July 4 and July 10 to be 7.6 and 26.8 mg. respectively, in 24 hours.

Three months later, on September 14, 1944, the patient wrote to the effect that she was losing her hair again, had pains and weakness in the legs, "bloated stomach," no signs of a monthly period and a great deal of distress on urination. Roentgen therapy was advised and given without any benefit. Her condition became gradually worse, and she returned to the Clinic on November 23, 1944. The blood pressure was then 202 systolic and 128 diastolic. There was intense acne of the face, chest and back. Her voice was like that of an adolescent boy. There was a pronounced fatty dorsal hump. In fact, practically all of the initial signs and symptoms of her illness had recurred and had increased in severity. One of the striking symptoms was extreme muscular weakness, particularly of the legs, which were so weak that she had to be assisted in stepping up on an ordinary hospital scale.

The change for the worse was also evidenced by the results of laboratory examinations listed under date of January, 1945, in Table 1. The very low concentrations of the plasma potassium and chlorides and the high values for the plasma sodium and carbon dioxide combining power are particularly significant. The daily urinary excretion of 17-ketosteroids and estrogenic substances was also greatly increased. Roentgenograms of the chest showed a discrete rounded shadow about 1½ inches (3.8 cm.) in diameter, which was thought to represent a metastatic lesion. Marked osteoporosis of the vertebrae was noted in the roentgenograms of the spine. Cultures of the urine showed organisms which were thought to be *Proteus ammoniae.*

The patient failed rapidly. On December 15, 1944, she vomited a large amount of blood and passed blood by rectum. There was another massive hemorrhage from the gastro-intestinal tract on January 17, 1945. She died the next day.

Necropsy report.—The following final pathologic diagnoses were made: Cushing's syndrome due to carcinoma of the left adrenal with recurrence after adrenalectomy; extension of recurrent adrenal carcinoma to the spleen, pancreas, stomach and diaphragm: metastasis to the regional and aortic lymph nodes, liver and lungs; atrophy of the right adrenal gland; basophilic degeneration of the pituitary gland; failure of luteinization of graafian follicles; active duodenal ulcer with hemorrhage and nephrolithiasis on the right.

The following observations were made: In the left upper part of the peritoneal cavity and in the expected position of the left adrenal gland, there was a soft, yellow, partly hemorrhagic nodular mass measuring 15 by 13 by 8.5 cm. (Fig. 2). This was considered to be a recurrence of the adrenal tumor previously removed. There were metastatic nodules in the liver, similar in appearance to the main tumor. The largest of these measured 4.5 by 3.5 by 3 cm. Metastatic lesions were also present in the upper lobe of the

right lung and in both lobes of the left lung. These ranged in size from 0.3 to 5 cm. in diameter.

The right adrenal gland was atrophic and weighed 1.5 Gm. (normal weight 6.7 Gm.)

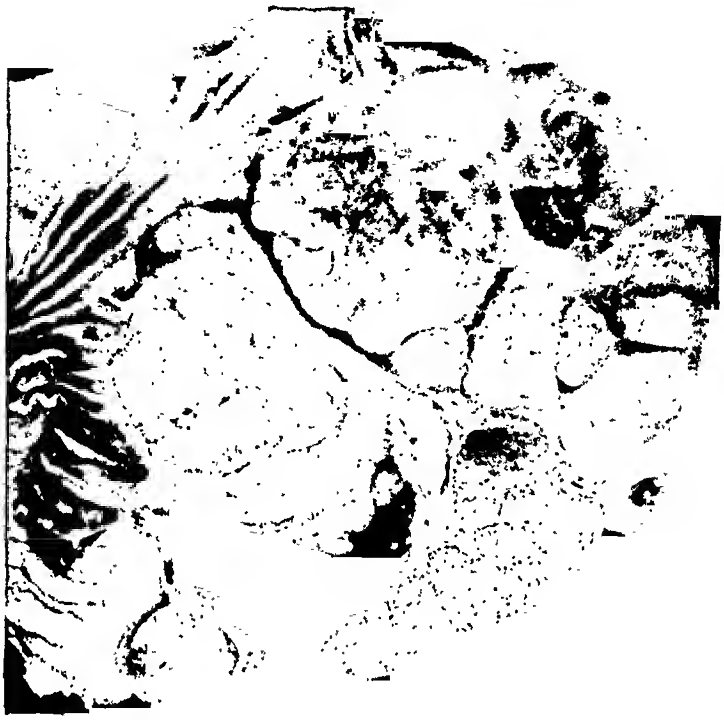

FIG. 2. Necropsy specimen. Recurrence of left adrenal cortical carcinoma with extension to spleen, stomach and tail of pancreas.

FIG. 3. Atrophic right adrenal gland (above) compared with a normal adrenal gland (below).

(Fig. 3). On inspection, the pituitary, thyroid and parathyroid glands appeared to be normal. The pancreas was grossly normal but its tail was adherent to the tumor. The ovaries appeared to be normal in size and appearance. The uterus measured 4 by 4 by 2.5 cm.

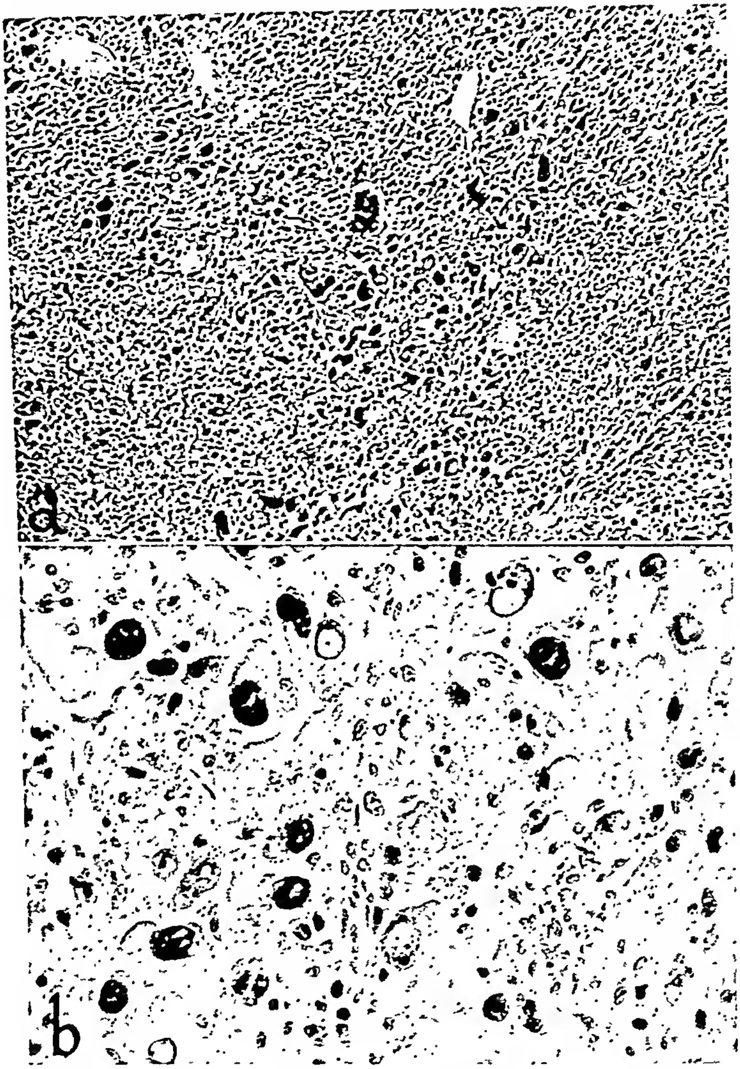

FIG. 4. Left adrenal cortical carcinoma showing pleomorphism and tumor giant cells (surgical specimen). *a.* ×57. *b.* ×220.

In the posterior wall of the duodenum, 2.5 cm. from the pylorus, there was an ulcer measuring 0.5 cm. in diameter which had eroded a small vessel and produced a fatal gastro-intestinal hemorrhage.

The right kidney weighed 280 Gm. In the pelvis there were three stones, measuring up to 0.8 cm. in diameter. The left kidney had been removed surgically.

The original left adrenal tumor, the recurrent tumor removed surgically, and the tumor found at necropsy were similar in appearance. All histologic sections of the left adrenal tumor and the recurrent lesions associated with it revealed an adenocarcinoma with cells resembling those of the adrenal cortex (Fig. 4*a* and *b*). The arrangement of these cells in cords and clusters with intervening blood vessels at times resembled the

zona glomerulosa and zona reticularis. More often, however, the cells were grouped in solid masses and bore no resemblance to any one zone of the adrenal cortex. The tumor was an adrenal adenocarcinoma graded 2 according to the method of Broders. Small areas of necrosis were present.

The individual cells revealed plemorphism. Many tumor giant cells were present. Foam cells were present in some areas. The average cell was 8 to 12 microns in diameter, but the diameter of the tumor giant cells ranged up to 40 or 50 microns. The nuclei were vesicular in many cells and in others, pyknotic. Their diameter in the average cell ranged from 6 to 8 microns, whereas in the tumor giant cells their diameter ranged up to

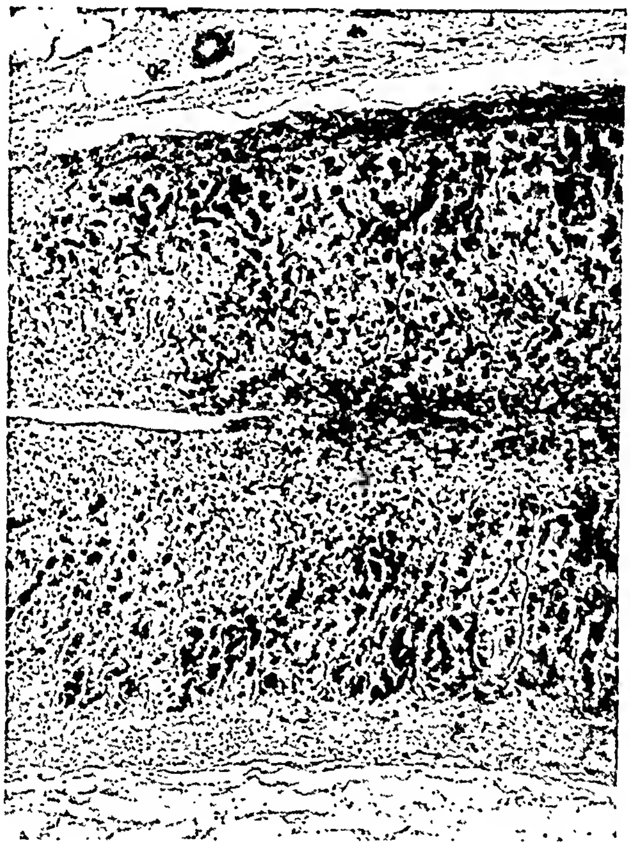

FIG. 5. Section of atrophic right adrenal gland. Entire thickness of the gland is shown (×57).

20 microns. The cytoplasm was finely granular, at times vacuolated, but for the most part, acidophilic. A mitotic figure could be identified in every fourth or fifth high-power field.

Sections of the tumor stained with Ponceau-fuchsin revealed the cytoplasm of many cells taking the fuchsin stain. Fuchsinophil granules were present in only a few cells. The Masson trichrome stain likewise revealed only a few cells with granules which took the stain. Sections stained with Sudan III revealed moderate variation of the fat content within the cells of the tumor. In most areas only small droplets were present within the cytoplasm of the tumor cells, but in areas with early necrosis, the droplets were more numerous and of larger size.

FIG. 6. Section of anterior lobe of pituitary body showing absence of granules in basophile cells, as well as hyalinization and vacuolization of the basophiles (Crooke's changes) (×320).

Marked atrophy of all three cortical layers of the right adrenal had occurred; this was especially noticeable in the zona glomerulosa (Fig. 5). An occasional cell in the cortex revealed small fuchsinophil granules after staining by the Ponceau-fuchsin method. With the Sudan III stain a much greater content of fat could be seen in the cortical cells of the right adrenal than within the cells of the tumor.

The metastatic lesions were histologically identical in appearance with the primary tumor, but showed larger areas of necrosis and infarction. Invasion of lymph nodes was common. In the pulmonary veins, tumor thrombi were identified.

The duodenal ulcer was typical of a benign, active peptic ulcer and had penetrated into the pancreas. In the kidney there was occasional hyalinization of glomeruli. The medium-sized arteries revealed a mild degree of intimal hyalinization with slight medial hypertrophy. Some of the tubules had clear vacuolated cytoplasm and calcium deposits were present in many of the tubules of the cortex. The findings in the kidney were consistent with the vascular change of benign nephrosclerosis.

In the anterior lobe of the pituitary body there were two small chromophobe adenomas, the largest of which measured 1 mm. in diameter. The majority of the basophilic cells revealed the changes described by Crooke. These consisted of absence of granulations, vacuolization and homogenous hyalinization of the cytoplasm (Fig. 6).

In the thyroid, involution was the predominant feature. The acini were all lined with low cuboidal epithelium and were filled with colloid. The parathyroids were histologically normal. The predominant cell was the chief cell, and oxyphil cells were sparse.

There was some persistent thymic tissue which contained numerous large degenerating Hassall's corpuscles. The pancreas was normal in appearance throughout.

In the ovaries there was a decrease in the expected number of primordial follicles for a person of twenty-three years. The graafian follicles showed development of granulosa cells, but there was no evidence of luteinization. Some of the follicles showed fibrosis around granulosa cells giving the appearance that corpora albicantia were developing without the intermediary phase of luteinization (Fig. 7*a* and *b*).

The endometrium of the uterus appeared to be in the midproliferative phase. The glandular cells were composed of low columnar or cuboidal endothelium and contained no mucus. No tortuosity of the endometrial glands was observed. The endometrium showed no histologic evidence of progestational effect.

Grossly and microscopically, the brain appeared to be entirely normal. The paraventricular hypothalamic nuclei were studied carefully and showed none of the changes described by Heinbecker in cases of Cushing's disease.

PROCEDURE FOR METABOLIC STUDIES

Metabolic studies were conducted during seven periods, consisting of five or six days each. The dietary content of sodium and chloride was kept constant throughout the entire study. During the first two periods the potassium content of the diet was kept at a fairly low level. A further reduction in the intake of potassium was made in periods 3 and 4. In period 5 the intake of potassium was increased to a high level by the administration of potassium chloride. The patient was then operated on, and a left adrenal cortical tumor was removed. Twenty-four days were allowed for the patient to recover from the immediate effects of the operation. At the end of this time metabolic studies were resumed. The intake of potassium

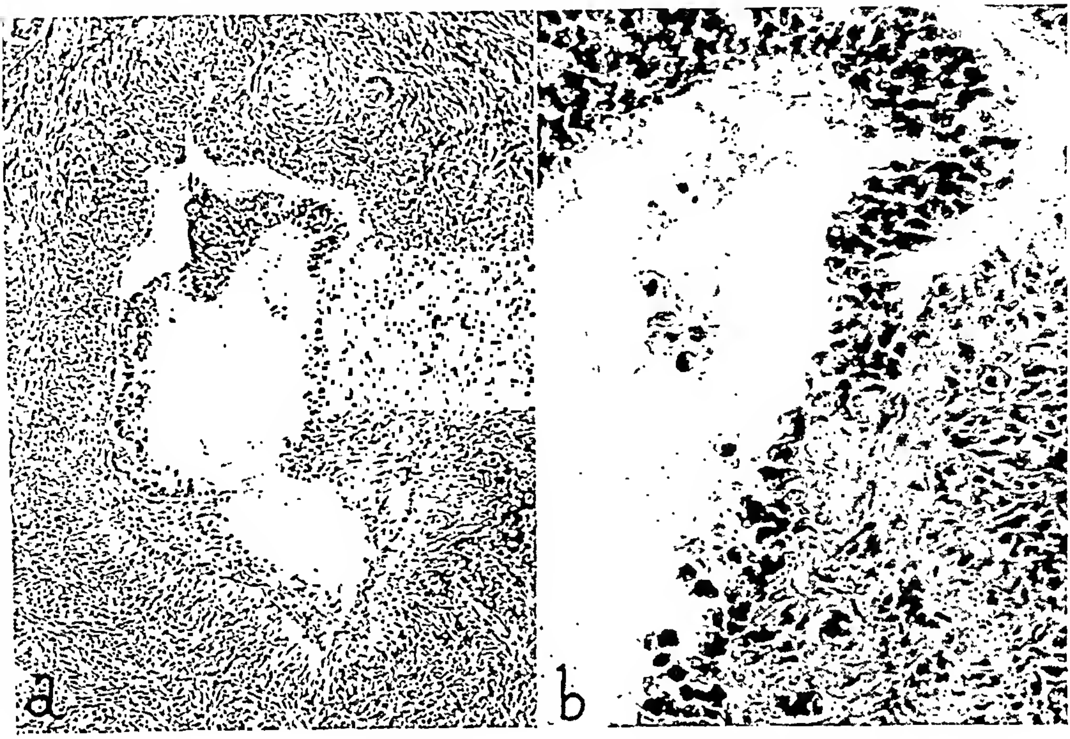

FIG. 7. Ovary showing graafian follicle with development of granulosa cells. The follicle shows beginning fibrosis, resembling formation of corpus albicans, with absence of luteinization. *a*. ×90. *b*. Details of granulosa cells (×350).

was again reduced to a very low level (periods 6 and 7) so that observations could be made and compared to the results obtained prior to the removal of the tumor.

Except for the changes made in the content of electrolytes, the diet was kept relatively constant throughout the seven periods. It supplied approximately 2,000 calories and contained approximately 200 Gm. of carbohydrate, 70 Gm. of protein and 100 Gm. of fat. The intake of fluid was also kept constant at 2,300 c.c. per day. In order to keep the intake of sodium and potassium at the desired level, food was cooked without salt and sodium chloride or potassium chloride was added. During periods 3 and 4, when the intake of potassium was greatly reduced, much of the food was cooked in parchment bags. The actual electrolyte content of the diet was determined by direct chemical analysis.

Analyses of the urine and stools were made for sodium, potassium, chloride, calcium and nitrogen. Urinary excretions of creatine and creatinine were also determined. At fairly regular intervals, blood was drawn for chemical studies.

TABLE 3. METABOLIC STUDIES IN A CASE OF CUSHING'S SYNDROME BEFORE AND AFTER REMOVAL OF MALIGNANT ADRENAL CORTICAL TUMOR

Period	Year, 1943	Days	Daily intake mEq. Na	Daily intake mEq. K	Daily intake mEq. Cl	Daily intake Gm. Ca	Daily intake Gm. P	Daily intake Gm. N	Balance* mEq. Na	Balance* mEq. K	Balance* mEq. Cl	Balance* Gm. Ca	Balance* Gm. P	Balance* Gm. N	Body weight, Kg.
1	Sept. 15–20	6	95	62	98	0.78	1.01	10.6	+12.2 (+2.0)	−24.1 (−4.0)	+67.1 (+11.2)	−0.53 (−0.09)	−1.47 (−0.25)	−13.5 (−2.3)	55.7 56.2
2	Sept. 21–26	6	95	62	98	0.78	1.01	10.6	−41.7 (−7.0)	−25.6 (−4.3)	−50.5 (−8.4)	+1.51 (+0.25)	+0.58 (+0.10)	−10.0 (−1.7)	56.1
3	Sept. 27–Oct. 2	6	106	46	104	0.75	1.02	10.9	+59.5 (+9.9)	−83.0 (−13.8)	+34.0 (+5.7)	−2.55 (−0.43)	−2.65 (−0.44)	−10.2 (−1.7)	55.3
4	Oct. 3–7	5	108	47	105	0.74	1.03	10.8	+70.0 (+14.0)	+39.0 (+7.8)	+44.9 (+9.0)	−0.31 (−0.06)	−0.03 (−0.01)	−2.0 (−0.4)	56.3
5	Oct. 8–12	5	103	126	184	0.77	1.02	10.9	−48.6 (−9.7)	+164.0 (+32.8)	+104.3 (+20.9)	+1.68 +0.34)	+1.81 (+0.36)	+6.4 (+1.3)	56.3
	Oct. 15	Operation													
6	Nov. 8–13	6	104	42	102	0.70	0.95	10.1	+205.0 (+34.2)	+101.0 (+16.8)	+181.7 (+30.3)	−0.53 (−0.09)	+0.74 (+0.12)	+30.5 (+5.1)	55.7 56.1
7	Nov. 14–19	6	104	42	102	0.71	0.86	9.3	+302.5 (+50.4)	+98.0 (+16.3)	+292.0 (+48.7)	−0.13 −(0.02)	+1.16 (+0.19)	+23.5 (+3.9)	56.5

* Balances are given as total amount per period and, in parentheses, as average amount per day.

Venous blood for chemical determinations was drawn under oil into a tube containing purified heparin. The carbon dioxide content of the plasma was determined by the method of Van Slyke and Neill (1); pH by means of the glass electrode method as described by Dill, Daly and Forbes (2); urea by the manometric method (3); sodium by the method of Butler and Tuthill (4); potassium by Hartzler's modification of the method of Shohl and Bennett (5); calcium by precipitation as oxalate from ashed plasma;

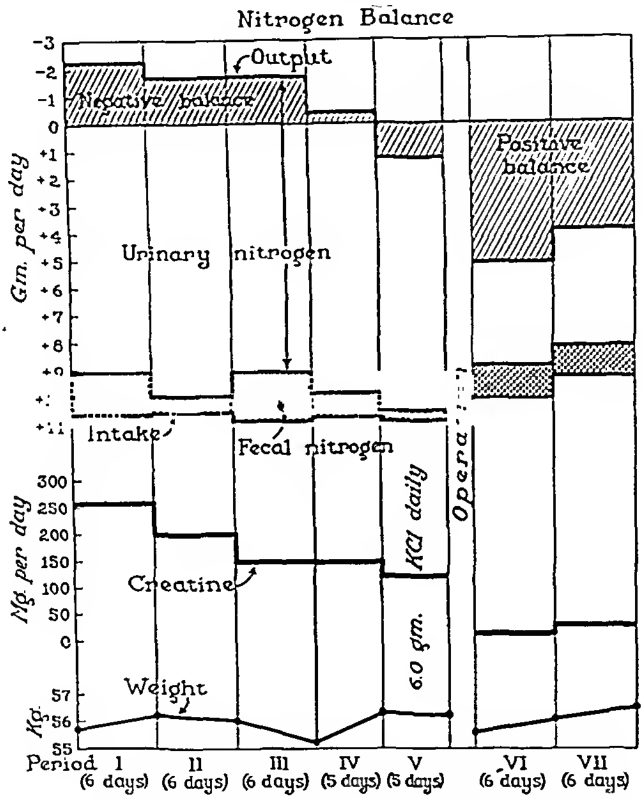

FIG. 8. Nitrogen balance, creatine nitrogen excretion and body weight before and after removal of malignant adrenal cortical tumor.

inorganic phosphorus by the method of Gomori (6); and chloride by a modification of the method of Keys (7).

In urine and in homogenized suspension of feces, chloride was determined by a modified Volhard-Harvey titration, and total nitrogen by the Kjeldahl method. Sodium, potassium, phosphorus and calcium were determined on ashed samples of these materials by the methods previously mentioned. Creatinine and creatine in urine were determined according to Folin (8), the latter by the open flask procedure.

RESULTS OF METABOLIC STUDIES

Results of the observations made are recorded in Tables 3, 4, 5, 6 and 7 and in Figures 8, 9 and 10.

Nitrogen.—Although the caloric value of the diet was adequate, the nitrogen balance was negative throughout the first four periods, during which the intake of potassium was at a low level (Table 3 and Fig. 8). The extent of the negative nitrogen balance tended to decrease as the low intake of potassium was continued. When the intake of potassium was increased sharply (period 5) a decrease in the urinary excretion of nitrogen followed and the nitrogen balance became positive. After the operation (periods 6 and 7) strongly positive nitrogen balances occurred under the same conditions which, prior to operation, were associated with a negative nitrogen balance.

Sodium, potassium and chloride.—During all of the first four periods, with the exception of period 2, the patient tended to retain sodium and chloride in approximately equivalent amounts even though the intake of these ions was not at a particularly high level. When the intake of potassium was increased in period 5 by the administration of potassium chloride, the urinary excretion of sodium increased, whereas the excretion of chloride decreased in spite of the administration of large amounts of chloride ion. In other words, on a low intake of potassium the sodium and chloride balance was, for the most part, positive; whereas after the administration of potassium chloride, sodium was lost from the body but chloride ion was retained.

The potassium balance remained negative during the first three periods and then became slightly positive. After the administration of potassium chloride, the potassium balance became strongly positive. After removal of the tumor, retention of sodium, potassium and chloride occurred. In general, it may be said that prior to the removal of the tumor a low intake of potassium was accompanied by a negative nitrogen and potassium balance and a retention of sodium. The chloride balance paralleled the sodium balance. When potassium chloride was administered, the body retained potassium and chloride but lost sodium.

The total balances of chloride, sodium, potassium and nitrogen before and after removal of the tumor are summarized in Figure 9.

Calcium and phosphorus.—In most respects, the balance of calcium and phosphorus paralleled the balance of nitrogen and potassium.

Data on intake and balance of sodium, potassium, chloride, calcium, phosphorus and nitrogen are given in Table 3. The balances of these substances before and after removal of the tumor are summarized in Table 4.

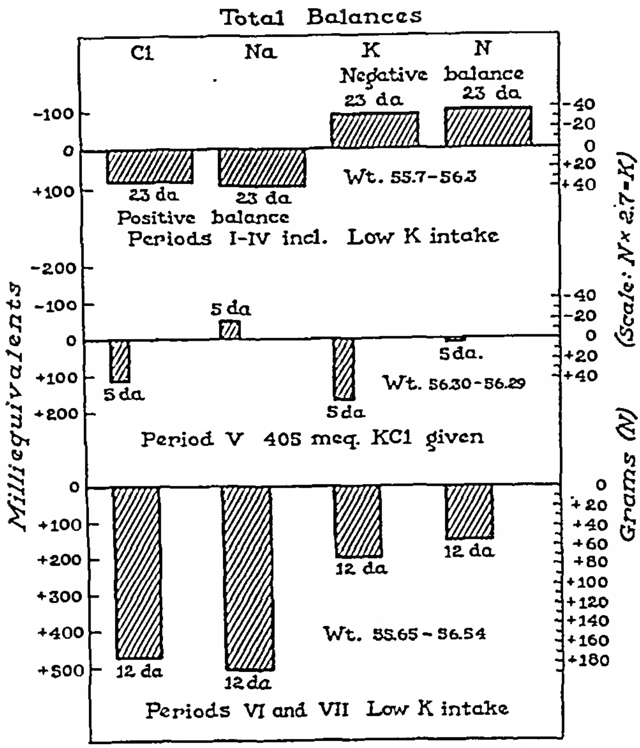

FIG. 9. Total balances of chloride, sodium, potassium and nitrogen before and after removal of malignant adrenal cortical tumor. The values represented by each column are the total balance of the substance in question for the number of days specified above or below the column.

TABLE 4. SUMMARY OF BALANCES IN A CASE OF CUSHING'S SYNDROME BEFORE AND AFTER REMOVAL OF MALIGNANT ADRENAL CORTICAL TUMOR

Period	Condition	Average daily balance					
		mEq.			Gm.		
		Na	K	Cl	Ca	P	N
1–4	Low preoperative intake of K	+ 4.4	− 4.1	+ 4.2	−0.08	−0.16	−1.55
5	High preoperative intake of K	− 9.7	+32.8	+20.9	+0.34	+0.36	+1.3
6–7	Low postoperative intake of K	+42.3	+16.6	+39.5	−0.06	+0.16	+4.5

Creatine and creatinine.—The excretion of creatinine remained at a rather constant level throughout the entire seven periods.

Creatine was present in the urine during the entire preoperative portion of the study. As the study continued, it decreased in amount. Postoperatively, it practically disappeared from the urine (Table 5 and Fig. 8).

Urinary pH and volume.—Throughout the first five periods the pH of the urine remained almost fixed at approximately 7.0 (Table 6). On four occasions it dropped to 6.5. After the administration of potassium chloride the urinary pH increased to 7.5 for the first two days, then fell back to its usual level, 7.0. After removal of the tumor, the highest value recorded

TABLE 5. AVERAGE DAILY URINARY EXCRETION OF CREATININE AND CREATINE BEFORE AND AFTER REMOVAL OF MALIGNANT ADRENAL CORTICAL TUMOR

Period	Year 1943	Days	Preformed creatinine nitrogen, mg.	Creatine nitrogen, mg.
1	Sept. 15–20	6	295	258
2	21–26	6	302	200
3	Sept. 27–Oct. 2	6	272	147
4	Oct. 3– 7	5	290	145
5	8–12	5	252	120
	Oct. 15	Operation		
6	Nov. 8–13	6	269	15
7	14–19	6	261	30

was 5.5 and during most of periods 6 and 7 the pH was reduced to 4.5 and remained relatively fixed at about that level.

Prior to operation, the daily urinary output varied from 1,275 c.c. to 2,250, averaging 1,700 c.c. After operation the average daily output was 980 c.c.; the range was from 400 to 1,600. Since the daily intake of fluid was kept at a constant level, the decreased output of urine which occurred postoperatively was indicative of retention of water. The administration of potassium chloride did not change the urinary output to any appreciable extent.

Plasma electrolytes.—The behavior of the plasma electrolytes is shown in Table 7 and Figure 10. It is apparent that during the periods in which the intake of potassium was kept at a low level, the concentration of the

TABLE 6. DATA ON THE VOLUME AND pH OF THE URINE

	Period						
	Preoperative					Postoperative	
	1	2	3	4	5	6	7
Volume Range, c.c.	1385–1990	1640–2250	1370–1875	1275–1690	1430–1880	805–1480	470–1160
Mean, c.c.	1712	1911	1555	1526	1619	1049	867
pH Range	6.5–7.0	6.5–7.0	6.5–7.5	6.5–7.0	7.0–7.5	4.5–5.0	4.5–5.5
Mean	6.8	6.7	6.9	6.9	7.2	4.6	4.7

TABLE 7. DATA ON PLASMA ELECTROLYTES

Period	Year, 1943	Days	mEq. per liter				Mg. per 100 c.c.		pH
			Na	K	Cl	CO_2	Ca	P	
1	September 15–20	6	133.9 136.0	4.4 5.2	94.5 96.7	32.0 33.0	9.8	1.8	
2	September 21–26	6	140.4	4.3	99.3	32.5	10.1	3.0	
3	September 27–October 2	6	142.6	4.2	94.5	34.7			
4	October 3–7	5	140.0	3.9	94.8	34.2	13.9	2.9	7.42
5	October 8–12	5	138.3	5.7	97.9	29.0	11.3	3.1	7.42 7.36
	October 15	Operation							
6	November 8–13	6	139.1 136.5	4.7 3.9	102.4 103.8	26.1 26.2	9.7 10.0	4.8 5.3	
7	November 14–19	6	140.0	3.9	104.4	25.3	9.2	5.3	

plasma potassium decreased progressively and was, for the most part, accompanied by an increase in the concentration of the plasma sodium. Simultaneously there occurred a slight rise in the carbon dioxide content of the blood plasma and possibly a slight decline in the level of the chloride. These tendencies were corrected by the administration of potassium chloride. In addition, when the intake of potassium was increased the pH of the blood decreased. These data taken alone are perhaps not of much sig-

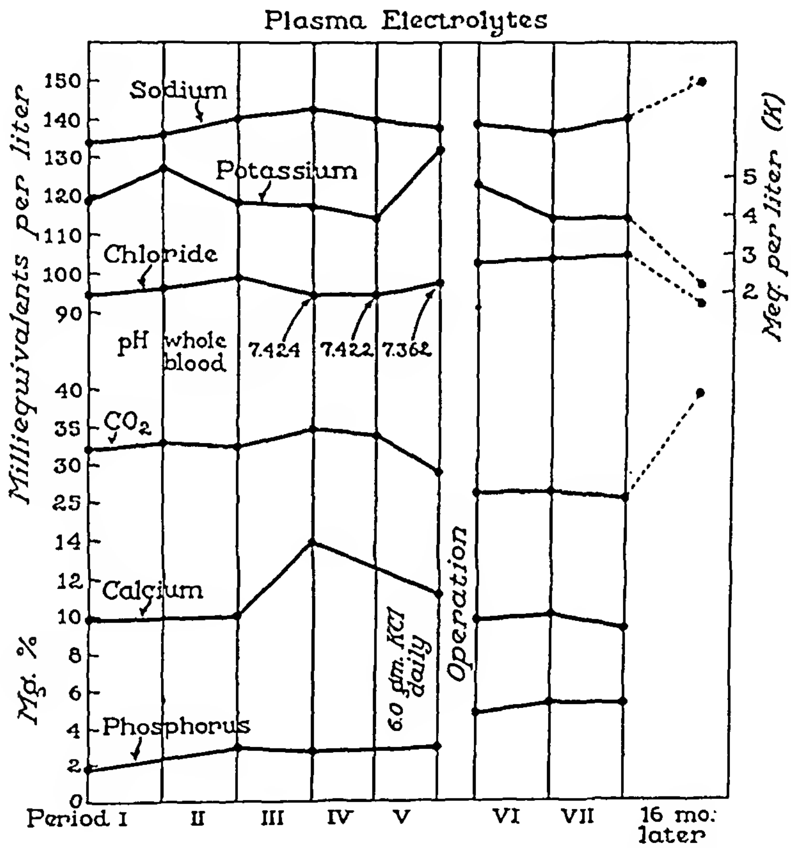

FIG. 10. Values for the plasma electrolytes and pH of whole blood during the several periods of study and after final recurrence of malignant adrenal cortical tumor.

nificance; but when they are compared with those we had obtained in previous studies on other patients (9, 10) and with those on the chemical status of the blood when the patient was again examined in January, 1945, their importance increases. It will be noticed that at the time the patient was studied in January, 1945, when the tumor had recurred, the hypokalemic hypochloremic alkalosis was thoroughly established and was accompanied by high values for the plasma sodium.

Urinary steroids.—Preoperative determinations of the urinary 17-ketosteroids gave values of 136, 125 and 132 mg. in 24 hours of which 71.5 per cent were 3(β)-hydroxy-17-ketosteroids. On the fourth day after oper-

ation these values fell abruptly to 2.4 mg. This level of excretion was maintained, with some fluctuations, until the patient was dismissed approximately six weeks later. Four months after dismissal, the quantity of 17-ketosteroids excreted was 50 mg. in 24 hours and in another two months it had increased to 107 mg. of which 60 per cent were 3(β)-hydroxy-17-ketosteroids. Immediately after the second operation the amount of 17-ketosteroids excreted was 7.6 mg. but in six days it had increased to 26.8 mg. Six months later it was 314 mg. in 24 hours.

One preoperative bio-assay for estrogen gave a value of 9,400 rat units in 24 hours. The values for the first seven days after operation were 1,700, 1,000, 100, 170, 120, 80 and 43 rat units respectively. A month later two normal values of 30 rat units were obtained. Six months after the second operation the quantity of estrogen excreted was 5,000 rat units in 24 hours.

The procedures for the isolation of the steroids from the urine in this case and the substances isolated have been described elsewhere (11) (case 5). The neutral fraction obtained from 43 liters of urine collected during a period of twenty-six days prior to operation weighed 9.3 Gm. Fractionation resulted in the isolation of 2,190 mg. of dehydroisoandrosterone, 192 mg. of etiocholan-3(α)-ol-17-one, 49 mg. of androsterone, 11 mg. of androstane-3(α), 11(β)-diol-17-one, 107 mg. of pregnane-3(α), 20(α)-diol, 55 mg. of Δ^5-androstene-3(β), 17(α)-diol, 10 mg. of Δ^5-androstene-3(β),16(β),17(α)-triol and 20 mg. of a substance with the empirical formula $C_{19}H_{32}O_2$.

The phenolic fraction contained 88,800 rat units of estrogen which was the equivalent of 177 mg. of estrone by the procedure used. Fractionation and isolation gave 62 mg. of estrone which represented 35 per cent of the total estrogenic activity. Estradiol and estriol could not be isolated.

COMMENT ON PATHOLOGIC ANATOMY.

Among the anatomic abnormalities noted at necropsy, the following perhaps are the most significant: 1) atrophy of the contralateral adrenal cortex, 2) Crooke's changes in the basophilic cells of the pituitary body, 3) chromophobe adenomas of the pituitary body, nephrolithiasis, nephrosclerosis, the absence of changes in the hypothalamus, and the failure of the graafian follicles to luteinize.

Contralateral adrenal cortical atrophy—Removal of benign or malignant functioning neoplasms of many of the endocrine glands is frequently followed by easily recognized symptoms suggesting reversible atrophy (disuse?) of the residual nontumorous endocrine tissue from which the neoplasm arose. The disastrous postoperative consequences of this atrophy in the case of adrenal cortical tumors have been repeatedly emphasized. Cahill (12) has expressed the opinion that acute postoperative adrenal

cortical insufficiency of severe degree is particularly likely to occur in cases of Cushing's syndrome, in contrast to its relatively infrequent occurrence in cases of adrenogenital syndrome. In our experience this generality seems to be true, although we still think it is advisable to treat preoperatively all patients having adrenal cortical tumor on the assumption that acute adrenal insufficiency may occur postoperatively.

"Crooke's changes" in the pituitary body.—Hyalinization of the basophilic cells of the pituitary body occurs regularly in cases of Cushing's syndrome and rarely in any other condition (13). This association holds true regardless of the presence or absence of recognizable adrenal cortical lesions. Crooke concluded that these changes were the anatomic accompaniment of a functional disturbance in the basophilic cells and that this disturbance was of fundamental significance in the etiology and pathogenesis of Cushing's syndrome. In a previous article (14) one of us (E. J. K.) presented a critical analysis of the significance of Crooke's phenomena and suggested that the changes in the basophilic cells were retrograde and perhaps degenerative in character. At this time we are merely calling attention to the difficulties in applying Crooke's concept to the case we have reported. In this case, if Crooke's contention and its implications are accepted, one almost has to conclude that an agent continually secreted by the hyalinized basophilic cells stimulated one adrenal cortex so intensely that neoplastic changes occurred, and simultaneously either caused or failed to prevent atrophic changes in the contralateral gland. One might explain the atrophy of the contralateral gland by assuming that the tumor grew because of the stimulus of pituitary adrenotropin, then became capable of hyperfunction independent of any tropic influence by the pituitary and finally inhibited the production of adrenotropin. On the other hand, rather than postulate excessive production of a pituitary tropic principle which was directly or indirectly responsible for the adrenal neoplasm and then try to reconcile the contralateral adrenal cortical atrophy with the postulate, it seems to us more reasonable to assume that the adrenal neoplasm grew and functioned *sui generis*, and in so doing caused the contralateral atrophy either directly or indirectly by inhibiting the production of pituitary adrenotropic material.

Associated chromophobe adenoma of the pituitary body.—Adventitious adenomas have been observed repeatedly in almost all of the endocrine organs, and the two tiny chromophobe adenomas that were found in our case possibly can be regarded as examples. On the other hand, it has been shown repeatedly in animals that the continuous administration of estrogens is followed by the appearance of adenomatous proliferation of chromophobe cells of the anterior lobe of the pituitary body (15, 16, 17). These changes occur in a species, namely, mice and rats, in which spontaneously

occurring hypophyseal tumors rarely are encountered. These observations, when taken in conjunction with the high urinary estrogenic content observed in our patient, make one suspect that the two chromophobe adenomas were not incidental but may have occurred as the result of the pathologic production of large amounts of estrogenic material by the adrenal tumor.

Nephrosclerosis and nephrolithiasis.—Renal calculi and renal colic are relatively frequent complications of Cushing's syndrome. This association is probably intimately related to the negative calcium balance and the consequential osteoporosis which is often an integral part of the syndrome. Albright (18) has pointed out that the negative calcium balance is in turn a manifestation of a negative nitrogen balance. According to him, negative nitrogen balance results in a loss of the protein matrix of bone, and this in turn is associated with a negative calcium balance, osteoporosis, calciuria and, in some instances, renal calculi and calcinosis.

Hypertension, one of the cardinal symptoms of Cushing's syndrome, might be followed by nephrosclerosis; conversely, nephrosclerosis conceivably might induce hypertension. (Selye (19) has produced severe experimental nephrosclerosis by the administration of desoxycorticosterone acetate.) Either of the two possible interpretations mentioned above might be applicable in our case.

Absence of follicular luteinization.—The histologic appearance of the endometrium was in keeping with, and reflected the histopathologic characteristics of the ovaries. The complexity of the endocrine disturbance present makes interpretation of the ovarian pathologic process practically impossible. Among the factors which would have to be considered are the simultaneous overproduction of both androgenic and estrogenic substances by the neoplasm and their retrograde effect on the pituitary body and its production of gonadotropic substances.

Hypothalamic lesions.—Degeneration of the hypothalamic nuclei and pathways has been noted in cases of Cushing's syndrome not associated with adrenal cortical tumor, and the possibility has been suggested that these degenerative lesions have etiologic importance (20). Our observations throw no light on this question.

COMMENT ON METABOLIC STUDIES

Since we are dealing in this report with only one case, the scope of our discussion concerning the alterations of metabolism in cases of Cushing's syndrome must be restricted. Certain features seem worthy of comment.

Nitrogen.—Prior to operation, the patient had a negative nitrogen balance, which was not great but which was, nevertheless, definite. Loss of nitrogen tended to decrease during the latter periods before operation.

This fact, in conjunction with Albright's (18) observations on the nitrogen balance in Cushing's syndrome, make one suspect that loss of tissue may occur intermittently,[1] or may first progress to a maximum, then decrease and finally cease entirely. Clinically, evidence indicative of loss of nitrogen-containing tissues is impressive in nearly all patients having Cushing's syndrome. After removal of the adrenal tumor there was a very strongly positive nitrogen balance. This dramatic postoperative retention of nitrogen is perhaps a better index of the antecedent loss of tissue than the preoperative balances indicate.

Administration of potassium chloride preoperatively was associated with a positive nitrogen balance. Usually loss of protein from the body is associated with loss of potassium; and conversely, synthesis of protein by the body is accompanied by retention of potassium. Is intracellular retention of potassium necessarily accompanied by retention of nitrogen? Can synthesis of protein take place in the body despite inadequate intake of potassium? Is the intake of potassium or the forced loss of potassium such as might occur in Cushing's syndrome or possibly in other disorders, ever a critical factor in the anabolism or catabolism of protein? We think these questions are worth consideration.

Our data throw no light on the interesting possibility postulated by Albright (18) that the adrenal steroids influencing carbohydrate and protein metabolism are antianabolic rather than catabolic in their action.

Creatine.—Creatine was found in the urine during the five preoperative periods. After removal of the adrenal tumor it practically disappeared. Creatinuria commonly occurs in association with wasting of muscle. In this case it can be regarded either as a manifestation of muscular wastage or as a specific effect of some substance produced by the adrenal tumor, or both. The decreased excretion of creatine in periods 3, 4 and 5 as compared with periods 1 and 2 is probably explainable on the basis of a decreased intake of creatine due to removal of this substance by dialysis during the cooking of meat in parchment bags for the very low potassium diet which was employed in periods 3, 4 and 5.

Sodium and potassium balances.—In general, sodium and chloride were retained preoperatively, whereas potassium was lost from the body. These are the phenomena that one might expect to occur if the adrenal cortical tumor was the source of excessive amounts of substances having properties resembling those of desoxycorticosterone acetate. They are also in keeping

[1] Fluctuations in intensity of the symptoms have occurred in several of our cases of Cushing's syndrome. In one, a complete remission occurred without treatment and lasted for about three months. The case reported by Josephson (21) is of particular interest in this respect. A comparable case has been observed by Albright (22).

with the tendency to become edematous, which these patients frequently exhibit.

Administration of potassium was followed by increased excretion of sodium and retention of potassium. Retention of potassium, however, exceeded loss of sodium. After operation, retention of sodium, chloride and potassium occurred. The retention of sodium and chloride was at first surprising and apparently not in keeping with preoperative trends. However, comparable retention of sodium and chloride has been observed in other conditions when negative nitrogen balances are suddenly corrected and rapid synthesis of protein presumably occurs. For example, emaciated diabetics, victims of starvation and patients having anorexia nervosa frequently become edematous following institution of appropriate therapy. These are other examples of the intimate relationship between the metabolism of nitrogen and electrolytes.

Total metabolism.—In spite of marked retention of sodium, chloride, potassium, water and nitrogen the patient did not gain any significant amount of weight postoperatively. By the usual methods of calculation she should have gained about 5 Kg. We have given a great deal of thought to this paradox and offer this tentative suggestion. Patients having Cushing's syndrome become fat while they lose muscle. The net result is usually gain in weight, although loss of weight may occur. In either event, changes in weight throw little light on what is taking place physiologically. If the pathologic physiology is corrected, gain in muscle and loss of fat should occur simultaneously. Here again, the scales may be misleading for if the two tendencies are approximately equal, weight would remain unchanged, although the appearance of the patient would change materially. On the other hand, if the weight of the fat lost was greater than the weight of the muscle gained, the net result would be a decrease in body weight. Clinically this latter phenomenon has been observed repeatedly after removal of adrenal cortical tumors. A comparable phenomenon frequently occurs during adolescence. The child eats ravenously, gains in height, loses its childish contours, becomes gangly and gains little weight, much to the concern of the apprehensive parent. Unfortunately, we did not make serial determinations of the basal metabolic rate or determine insensible loss of water. Postoperatively, there was a significant increase in the basal metabolic rate, from 0 to 15 per cent, and a decrease in the respiratory quotient, from 0.83 to 0.74. Both of these observations are in keeping with the explanation we have suggested.

Urinary pH.—We have been impressed by the frequency with which patients having Cushing's syndrome excrete urine that is alkaline to litmus and also by the frequency with which infections of the urinary tract occur. The relatively high pH of the urine prior to operation and the sharp de-

crease in the pH which occurred postoperatively might well be reflections of the disordered metabolism of sodium and potassium and the tendency for alkalosis to develop.

Plasma electrolytes.—In previous studies it was shown that the administration of potassium chloride corrected the hypochloremic, hypokalemic alkalosis of Cushing's syndrome. It was logical to infer that restriction of potassium would make it worse. Our data tend to support this inference. When first seen, the patient had a mild alkalosis. Restriction of potassium was accompanied by a slight depression of the concentrations of plasma potassium and chloride and slight increases of the values for plasma sodium and carbon dioxide. This tendency was corrected by the administration of potassium chloride. After removal of the adrenal tumor the plasma electrolyte pattern was normal even though the intake of potassium was restricted. Sixteen months later, when the tumor had recurred, the intensity of the alkalosis was as severe as we have seen in cases of Cushing's syndrome.

Urinary steroids.—In most respects, the quantity and variety of steroids isolated from the urine resembled those that have been found in similar cases. Excretion of 17-ketosteroids was increased and there was a high concentration of the beta fraction, chiefly because of the presence of a large amount of dehydroisoandrosterone. The large amount of estrogenic material that was isolated is of considerable interest. Estrogenic substances have been found in a few cases of adrenal cortical tumor and sometimes, but more often not, in conjunction with feminizing syndromes. The simultaneous excretion of estrogens and steroids that have androgenic properties is unusual but not surprising. It again emphasizes the protean functional capacities of adrenal cortical neoplasms. Nearly thirty adrenal steroids have been isolated from the adrenal glands of animals. Some of these influence the metabolism of salt and water, others influence the metabolism of carbohydrate and protein, while 11-dehydrocorticosterone (compound A) or others may influence metabolism of fat and still others have androgenic, estrogenic or progestational properties. The variety of clinical pictures, the quantitative and qualitative variation in the urinary steroids and the absence of histologic homogeneity in the tumor all tend to support the thesis that these neoplasms can produce in excessive amounts almost any one or all of normal or even abnormal adrenal steroids.[2]

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[2] For a more detailed discussion of the mechanism by which the pathologic physiology of Cushing's syndrome might be produced by an excess of adrenal steroids, the reader is referred to articles by Kenyon (23), Albright (18), and Kepler and associates (24).

COMMENT ON THE PATHOGENESIS OF CUSHING'S DISEASE AND CUSHING'S SYNDROME.

Although we are reporting a case of adrenal cortical tumor associated with Cushing's syndrome, one of us (E. J. K.) is taking this opportunity to discuss the pathogenesis of Cushing's disease. Before proceeding further, a few historical remarks are pertinent.

Most of the early efforts to account for the clinical picture of Cushing's disease emphasized the role of the anterior lobe of the pituitary body. It was assumed that adenomatous basophilic cells produced excessive amounts of various pituitary hormones and that these in turn were responsible for the clinical symptoms, either by stimulating other members of the endocrine system or by acting on other loci in the body. This view was strengthened when it was shown that roentgen therapy to the pituitary body sometimes was followed by a remission of symptoms. Later observations discredited the part played by the adenomatous basophilic cells and incriminated the cells described by Crooke.

When it became apparent that the essential features of the syndrome were not restricted to cases of basophilic tumors of the pituitary body but could occur in association with any one of several pathologic lesions, particularly adrenal cortical hyperplasia and adrenal cortical tumor, another school of thought developed. This school emphasized the importance of the adrenal cortices. Some of its members maintained that the disease began as a primary disorder of the adrenal cortices. Others held the view that the primary disturbance began elsewhere, usually in the pituitary body but that most, if not all, of the endocrine symptoms in all cases of Cushing's syndrome were an expression of disturbances, hyperfunctioning in character, of the adrenal cortices. Their position was greatly strengthened after it was shown that when Cushing's syndrome was associated with adrenal cortical tumor, surgical removal of the tumor was followed by a prompt and dramatic remission of the endocrine symptoms. There was, nevertheless, one very formidable objection to their theory. Since adrenal cortical insufficiency was characterized by disturbances in the metabolism of electrolytes, why did not the hyperfunctioning adrenal cortical lesions also disturb the electrolyte pattern of the blood? In due time, this objection was answered by the appearance of a number of cases of Cushing's syndrome in which there were associated electrolytic abnormalities. True, the abnormal electrolyte pattern was not exactly the antithesis of that which was found in Addison's disease; but it was sufficiently antithetic in character to make one suspect that it was the result of excessive adrenal cortical activity. The first group of these examples consisted almost entirely of cases of adrenal cortical "hyperplasia." There were in the group,

however, some cases in which the adrenal cortices were reported to be grossly and microscopically normal; consequently, it could be argued that the abnormal electrolyte pattern of the blood was not necessarily the result of adrenal cortical hyperfunction. Subsequently, cases were discovered in which the hyperfunctioning adrenal lesion was proved to be an adrenal cortical tumor.[3] These cases of adrenal cortical tumor are particularly important in that they established beyond any reasonable doubt that the disturbance in electrolytes was of adrenal origin. In the case we have reported in this paper, the evidence is particularly convincing because the chemical condition of the blood each time became normal after the two removals of the tumor and each time became abnormal again after the two recurrences. In a sense, then, these cases of adrenal cortical tumor can be regarded as a missing link, so that now one can state with finality that all of the endocrine symptoms which thus far have been reported to occur in cases of Cushing's disease have also been found in cases of adrenal cortical tumor.

If adrenal cortical hyperfunction can account for all of the symptoms of Cushing's disease, what part does the anterior part of the pituitary body play in the pathogenesis of this condition? At least two possibilities come to mind. First, one can assume that for some unknown reason basophilic hyperfunction occurs. This hyperfunction is characterized by the overproduction of an adrenotropic agent which stimulates the adrenal cortices. The anatomic response to this stimulation is adrenal cortical hyperplasia or neoplasia; the functional response is adrenal cortical hyperfunction. Note that this assumption seems to imply that the basophilic hyperfunction is limited in scope and restricted in action to the overproduction of adrenotropic substance alone, for the reason that if more than one type of tropic agent were produced some endocrine symptoms would persist after the hyperfunctioning adrenal cortical neoplasm had been removed. This implication in turn results in either one of two others: (a) normal basophilic cells are able to make several hormones but in Cushing's syndrome their function is disturbed so that they make only one, an adrenotropic substance, in excess; or (b) normal basophilic cells can make only one tropic agent and of this one, in Cushing's syndrome, they make too much. Neither of the alternatives is particularly satisfying. One dislikes the first because in what is probably an analogous situation, eosinophilic pituitary hyperfunction (acromegaly) usually seems to be associated with the overproduction of several pituitary principles such as the growth hormone, thyrotropic hormone and the diabetogenic hormone. These, in turn, are

[3] One is the case which we have reported herein; another we observed but did not report. Albright (22) encountered a third case and others are mentioned in the literature. (23, 25).

responsible for the continued growth, the thyroid enlargement and the diabetes that go with this condition. One dislikes the second alternative because it seems to relegate the production of all the hormones of the anterior part of the pituitary body, except the adrenotropic, to the eosinophilic cells.

With regard to the second possibility, as to the part played by the anterior lobe of the pituitary body, one can assume that for some unknown reason the adrenal cortices become hyperplastic or neoplastic and hyperfunction; but that this reaction cannot take place unless the anterior part of the pituitary body is reasonably intact functionally. According to this view the pituitary body does not instigate the disorder but merely provides a set of conditions without which the real etiologic agent cannot be effective. This distinction between a necessary condition and a prime etiologic factor is a bit slippery, but it can be exemplified easily. Before the micro-organism responsible for pulmonary tuberculosis was discovered, inanition, bad air and overcrowding seemed to be the "cause" of tuberculosis because when these factors were eliminated the epidemiologic characteristics of the disease could be practically eliminated from a community. Now it is apparent that these "predisposing" factors do not "cause," but merely provide conditions which are necessary or favorable for the dissemination of the tubercle bacilli and its growth and multiplication in the affected host. Likewise, in the case of Cushing's syndrome (or for that matter, diabetes mellitus and exophthalmic goiter) there is the logical possibility that the anterior lobe of the pituitary body merely provides a set of circumstances that are necessary for the development of the syndrome. If this were the case, therapeutic radiation or any other form of therapy which would alter or reduce the function of the anterior part of the pituitary might be followed by remission or favorable alteration of the symptoms.

One of the foundations of the pituitary theory of Cushing's syndrome is the frequency with which basophilic tumors of the pituitary body are found. This high incidence cannot be dismissed lightly and requires for its interpretation something more than a philosophical "brushoff." Cushing (26) reasoned that since eosinophilic tumors hyperfunction and cause acromegaly, basophilic tumors might also hyperfunction and cause "basophilism." His concept was contested on two grounds: first, many of the tumors were so small that they escaped detection unless special search was made for them; second, basophilic tumors often were found in cases in which none of the symptoms that he had described was present. Neither of these objections is valid. Cushing disposed of the first, for the most part, by calling attention to the fact that size *per se* of an adenoma is no indication of its functional potentialities. This is apparent in the case of tiny hyperfunc-

tioning parathyroid tumors or islet cell tumors of the pancreas. The second objection, based on frequency of occurrence, likewise carries little weight, since it is now well known that, generally speaking, symptomatically silent endocrine tumors are found at necropsy much more frequently than are tumors which have been proved to cause symptoms.

If the objections based upon size of a tumor and the frequency with which nonfunctional tumors occur can be dismissed as being insignificant, what further objections, other than those that can be raised from purely clinical considerations, are there to the original pituitary theory and its various modifications? There is one objection, and that one is largely inferential in character; namely, that development of basophilic tumors, as well as the Crooke's changes, might be a retrograde phenomenon. Elsewhere in this paper, attention was called to the fact that chromophobe tumors of the anterior lobe of the pituitary body have been produced in animals by the prolonged administration of estrogenic substances. If this is the case, it is not illogical to suspect that administration of some of the adrenal steroids might be followed by the appearance of basophilic tumors. There is some evidence, not conclusive to be sure, that bears on this point. Woolley (27) in a personal communication to one of us (E. J. K.) has said that the experimental production of tumors of the adrenal cortex has been followed by the appearance of basophilic adenomatous changes in the anterior lobe of the pituitary body. He, however, was not altogether satisfied with the cytologic examinations that were employed. Furthermore, the tumors of the adrenal were induced in gonadectomized mice, and consequently, there was the possibility that the adrenal tumors might have been induced by the well-known changes in the function of the anterior lobe that occur after the gonads have been removed.

The pathogenesis of Cushing's disease involves more than an intellectual challenge. This disease, in our experience, is one of the most difficult and often one of the most unsatisfactory of all endocrinopathies to treat, and all types of treatment employed or recommended have been bases entirely on hypotheses. Even when the syndrome is associated with an adrenal cortical tumor, the satisfaction that goes with brilliant immediate cure is too often short-lived because of a fatal recurrence of the tumor or metastasis to the liver or lungs. If these disorders actually begin as a disturbance of the adrenal cortices, it seems illogical and unnecessary to subject the patient to roentgen therapy to the anterior lobe of the pituitary body, particularly when the therapeutic results are often disappointing and uncertain. If the fundamental anatomic site of the disorder is in the pituitary body, therapy should be directed toward depressing its adrenotropic function by any means now available or yet to be discovered, not only in cases of adrenal cortical hyperplasia, but even in cases of adrenal cortical tumor after the tumor has been removed.

At the time of this writing in 1947, the question of the adrenal versus the pituitary theory of Cushing's syndrome is still open. The problem might be approached experimentally from at least two angles. Patients with Cushing's syndrome might be studied for the content of adrenotropic substance in the blood and urine after thoroughly reliable methods become available. Some efforts along this line have been made. There is still some room for skepticism, however, regarding the conclusions which are based on the methods now employed. If the condition is characterized regularly by a high concentration of adrenotropic substance in the blood and urine one might well conclude that among the endocrine organs the anterior lobe of the pituitary body is primarily at fault. On the other hand, if the administration of adrenal steroids, particularly those steroids which affect the metabolism of protein and carbohydrate, such as compound E (11-dehydro-17-hydroxycorticosterone), should be followed in suitable experimental animals by the appearance of Crooke's changes or basophilic tumors or both, in the anterior lobe of the pituitary body, it would seem that the adrenal cortices are primarily at fault and that the pituitary abnormalities are secondary or at least incidental and could be ignored therapeutically.

SUMMARY

A case of adrenal cortical tumor has been reported. The patient presented the clinical features of Cushing's syndrome, including hypertension and hypokalemic, hypochloremic alkalosis. Urinary excretion of both 17-ketosteroids and estrogenic substances was increased to abnormally high levels. The beta fraction of the urinary 17-ketosteroids was found to be increased. Remission of symptoms followed the removal of the tumor. Recurrence of the tumor was accompanied by the reappearance of the clinical picture. Removal of the recurrent tumor was followed by a temporary and incomplete remission of symptoms. Recurrence of the tumor and the appearance of metastatic lesions in the lungs were associated with the symptoms that were present prior to the removal of the tumor. Roentgen therapy proved to be ineffective. At necropsy the following significant observations were made: the anterior lobe of the pituitary body contained two small chromophobe tumors; Crooke's changes were found to be present in the basophilic cells of the anterior lobe; the hypothalamus appeared normal; the tumor had recurred locally and had metastasized to the lungs; the contralateral adrenal cortex was found to be atrophic; although the patient had osteoporosis, the parathyroid bodies appeared to be normal; and the endometrium presented evidence of an apparent absence of progestational effects. The immediate cause of death was a bleeding duodenal ulcer.

A large quantity of urine was collected before operation and various steroidal compounds were isolated and identified. These included dehydroisoandrosterone; etiocholan-3(α)-ol-17-one; androsterone; androstane-

3(α), 11(β)-diol-17-one; pregnane-3(α),20(α)-diol; Δ^5-androstene-3(β),17(α)-diol; Δ^5-androstene-3(β), 16(β), 17(α)-triol; estrone and a substance with the empirical formula $C_{19}H_{32}O_2$.

Metabolic studies were conducted before the first operation and shortly thereafter. Under the conditions employed, the over-all nitrogen balance before operation was negative. Creatine was present in the urine. Potassium was lost from the body and sodium was retained when the potassium content of the diet was kept at a low level. Administration of potassium chloride tended to correct the alkalosis, the negative balances for potassium and nitrogen and the positive balance for sodium. After removal of the tumor, nitrogen, sodium, potassium and chloride all were retained in large amounts, and creatinuria practically disappeared. The amount and pH of the urine decreased postoperatively. The metabolic data suggested that the functioning adrenal tumor caused loss of muscle and deposition of fat and that its removal was followed by loss of fat and deposition of muscle.

The pituitary versus the adrenal theory of the pathogenesis of Cushing's disease has been discussed, and clinical and experimental methods which might eventually prove helpful in establishing or refuting one or the other of these theories have been suggested.

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THYROTOXICOSIS FACTITIA (ALIMENTARY THYROTOXICOSIS)

ITS DIFFERENTIATION FROM SPONTANEOUS THYROTOXICOSIS WITH THE AID OF RADIOACTIVE IODINE

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THE clinical picture of self-induced hyperthyroidism may be indistinguishable from that of spontaneous thyrotoxicosis but the history usually makes the differential diagnosis possible. However, some investigators have pointed out clinical symptoms by which they feel one should be able to differentiate between the two conditions. Falta (1) stated that eye signs never were present in simple hyperfunction of the thyroid. Several later investigators have been of the same opinion. Nevertheless patients with alimentary hyperthyroidism may present slight eye signs of the type seen in spontaneous thyrotoxicosis, particularly if they have previously had true Graves' disease. On the other hand, patients with endogenous thyrotoxicosis may show minimal or no eye signs. The eye signs are, therefore, not dependable in the differential diagnosis.

Enlargement of the thyroid gland is usually observed in endogenous thyrotoxicosis, but occasionally palpation of the thyroid reveals no enlargement. On the other hand, patients with induced hyperthyroidism, in whom one would expect a gland of normal size, may have an enlargement due to a nontoxic goiter. Therefore one cannot make the differential diagnosis by the physical examination. In most cases of alimentary thyrotoxicosis, however, the diagnosis can be made from the history of an excessive intake of thyroid, usually in an effort to reduce weight. With cessation of the thyroid medication the symptoms will subside in a few weeks and the diagnosis can be confirmed.

One of the greatest difficulties in the differential diagnosis between these two conditions is encountered with patients having psychiatric problems, who will not admit that they are taking thyroid.

Perkin, McFarland and Hurxthal (2) and Hurxthal (3) have studied a number of patients with secretly self-induced hyperthyroidism and found that urinary iodine determinations were of great help in establishing the

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diagnosis. In normal individuals the urinary output of iodine amounted to approximately 100 to 150 micrograms per day and in patients with spontaneous hyperthyroidism the excretion was approximately 250 micrograms per day. However, if the thyrotoxicosis was induced by thyroid medication of 0.3 Gm. or more of desiccated thyroid daily, the urinary output of iodine was still higher.

Accurate diagnosis of thyroid disorders has been greatly facilitated by the use of serum protein-bound iodine determinations as an index of circulating thyroid hormone and of tracer doses of radioactive iodine as an index of the avidity of the thyroid gland for iodide. The protein-bound iodine of serum is almost invariably elevated in thyrotoxicosis [Salter, Bassett and Sappington (4); Winkler, Riggs, Thompson and Man (5)].

In connection with the problem of differential diagnosis it is especially interesting to note that Riggs, Man and Winkler (6) found that the administration of large quantities of dried thyroid to normal subjects produced a clinical picture of hyperthyroidism with most of the signs and symptoms of spontaneous hyperthyroidism, including an elevation of both serum-precipitable iodine and basal metabolic rate.

Tracer doses of radioactive iodine can be used for diagnostic purposes as was first demonstrated by Hamilton and Soley (7). Keating, Power, Berkson and Haines (8) found that the urinary excretion of tracer doses of radioactive iodine was less than normal in hyperthyroid patients. In a clinical study by McArthur, Rawson, Fluharty and Means (9), it was demonstrated that a low urinary excretion of radioactive iodine was helpful in establishing the diagnosis of Graves' disease, and that a high excretion aided in excluding this diagnosis. Skanse (10) has demonstrated that under the standardized conditions employed, the 48-hour urinary excretion of tracer doses of radioactive iodine in 25 patients with thyrotoxicosis varied between 6 and 32 per cent as compared to an excretion of 53 to 84 per cent in 15 euthyroid subjects.

We have recently observed two patients, one of whom certainly, and the other most probably, developed hyperthyroidism due to clandestine ingestion of thyroid. Since the case histories are very characteristic of this category of patients it has seemed worthwhile to present them as illustrations of the use of serum protein-bound iodine determinations and especially of the urinary excretion of radioactive iodine in the differential diagnosis between alimentary and spontaneous hyperthyroidism.

CASE REPORTS

Case No. 552354. The first patient, M.McM., a 37-year-old unmarried graduate nurse, was referred to this hospital for study of recurrent symptoms of thyrotoxicosis.

Her first hospital admission (to an outside hospital) was on January 15, 1936. At that time she presented a characteristic picture of thyrotoxicosis. Her weight was 50 Kg., her pulse rate was 110 to 120 and her basal metabolic rate (BMR) was between plus 30 and plus 40 per cent. Her thyroid was bilaterally enlarged. She reported a history of urinary frequency since December 1935. She was thirsty and had urinary volumes up to 4000 cc. a day.

She was prepared for operation with iodine (Lugol's solution 10 drops three times a day). Subtotal thyroidectomy was performed on January 28, 1936, and only small strips of thyroid were left on each side. The pathological report was one of "hyperplastic goiter." Her postoperative condition was fairly satisfactory but her pulse rate remained elevated. A basal metabolic rate, taken eighteen days after operation, was plus 8 per cent. A diagnosis of diabetes insipidus was made. Pituitrin was given in an attempt to control urinary frequency but it had little effect. She was discharged on February 26, 1936.

The patient felt quite well until 1940, when she again developed almost the same symptoms as in 1936 with intolerance to heat, muscle weakness, easy fatiguability and nervousness. She was still suffering from thirst and polyuria. She was admitted to the same hospital on October 28, 1940, presenting a picture of typical thyrotoxicosis. Her weight was 41.5 Kg., pulse 100 to 110, and her BMR's were plus 32 and plus 25 per cent. Her thyroid showed a slight bilateral enlargement in the region of the upper poles.

She was again operated upon and a small mass of thyroid tissue (4 by 3 by 3 cm.) was removed from the region of the right upper pole and a similar mass (2 by 2 by 1 cm.) from the left upper pole. The pathological report was again one of "hyperplastic goiter." Her BMR thirteen days after operation was minus 14 per cent.

On January 2, 1941, the patient claimed that she developed a severe headache followed two hours later by convulsions. She was given calcium gluconate intravenously for several days, 20 units of parathormone daily for seven days and then calcium by mouth until May 5, 1941. Following this episode of convulsions her polydipsia and polyuria stopped abruptly and these symptoms have not recurred.

On July 4, 1941, she was again examined. Her BMR was minus 28 per cent and her blood calcium was 9 mg. per cent. Because of the symptoms of hypothyroidism she was given 130 mg. of desiccated thyroid daily for one month and 65 mg. daily for the two following months. This seems to have been the first period during which she took thyroid. She seemed to feel well until the beginning of 1943, when she again began to feel tired and nervous . A BMR on January 31, 1943, was plus 42 per cent. She was readmitted to the hospital on March 1, 1943, and x-ray therapy of her neck was started. She had 5 treatments during two weeks, after which she developed radiation sickness and symptoms of tetany. She was treated with large amounts of calcium, parathormone and sedatives and recovered after about ten days. Blood calcium, chlorides, phosphorus and CO_2-combining power were all within normal limits at the time of the attack of tetany. Her BMR's were falling (March 22, plus 13 per cent; March 30, plus 11 per cent; April 29, minus 4 per cent; May 1, minus 12 per cent) and her thyrotoxic symptoms abated to some degree.

She felt better for some time but in the fall of 1943 she again became very nervous and restless. She entered the hospital again November 1, 1943. At that time her BMR's were plus 46 per cent and plus 39 per cent, and her pulse rate was about 150. She was operated upon for the third time on January 4, 1944. The thyroid region was explored and a piece of tissue was removed which was thought to be thyroid, but the pathological report showed that it was thymus gland. While she was in the hospital her BMR fell to minus 6 per cent but the patient did not notice any substantial improvement. She was discharged on April 3, 1944.

In May 1944 thiourea was started, but toxic symptoms appeared ten days later, so thiouracil was substituted in a dose of 0.6 Gm. daily. After six weeks the BMR was minus 20 per cent so the dose was reduced to 0.4 Gm. daily for one month and then stopped. She felt better for a few months but later the symptoms of hyperthyroidism recurred. She was again treated with thiouracil, 0.6 Gm. daily, for about six weeks (in the spring of 1945).

Readmitted to the hospital June 16, 1945, she was still restless and nervous and had a BMR of plus 33 per cent. She was treated with Lugol's solution, high caloric diet and sedatives. This therapy appeared to have very little effect on her symptoms and on July 9 she was discharged for a prolonged rest at home on the same regimen. However, she, did not improve at all, and on September 11, 1945, she was admitted again with symptoms of irritability, restlessness and nervousness, weakness, heat intolerance, sweating and widening of palpebral fissures. Her BMR was plus 88 per cent. Several examiners found no evidence of goiter. There was no enlargement of the sella turcica, no tumor in the pelvis and no evidence of tumor of the adrenals. Thiouracil, up to 1.6 Gm. a day, did not have any effect on the symptoms of thyrotoxicosis. Her BMR on January 24, 1946, was plus 64 per cent. She was seen by a psychiatrist but there did not seem to be any psychiatric or psychological aggravating factors. The patient was discharged on March 24, 1946. She was then seen several times by her doctor. All signs and symptoms of hyperthyroidism persisted. (The history so far is based upon correspondence we had with the hospital where she was treated before the admission to the Massachusetts General Hospital.)

She was admitted to the Thyroid Clinic of the Massachusetts General Hospital on October 28, 1946, for study. At that time her history in summary revealed: A 37-year-old woman who had developed thyrotoxicosis ten years before, since which time she had had three thyroid operations (1936, 1940 and 1944), x-ray treatment (1943) and therapy with thiouracil and iodine—all without permanent relief of her symptoms of hyperthyroidism. The examination of the patient showed a thin hyperactive individual with warm, moist skin, scars in the neck after thyroid surgery, and without palpable thyroid tissue. Her eyes were slightly prominent with a stare, lid lag, and globe lag. There was a marked, coarse tremor of the hands. Some slight right facial paralysis was present but Chvostek and Trousseau signs were negative. Her weight was 44.3 Kg. and her BMR was plus 51 per cent. Her pulse was rapid and regular, varying between 110 and 150, and her blood pressure was 130/170. Urine-analysis findings were normal and routine blood counts and differential smears were within normal limits. Pelvic examination revealed a slightly enlarged right ovary, somewhat firmer than normal; her left ovary was not definitely palpable.

Thus the patient presented a clinical picture that could be explained by an overproduction of calorigenic hormone which might be either thyroid hormone or adrenalin. We thought the following possibilities had to be considered:

1. Genuine recurrent thyrotoxicosis (Graves' disease). With no thyroid tissue palpable in the neck and the right ovary somewhat enlarged, we had to consider the possibility of struma ovarii as responsible for her thyrotoxicosis.

2. Hyperadrenalinemia due to phaeochromocytoma. The history and the earlier course of her disease were definitely against such a diagnosis. The patient did not have hypertension, nor had there been observed any par-

oxysmal episodes of hypertension despite prolonged hospital observation. A phaeochromocytoma as a cause of her symptoms of many years' duration should also have produced some enlargement of the heart or other signs of heart stress. The diagnosis of phaeochromocytoma therefore was not considered to be very likely.

3. Thyrotoxicosis factitia (self-induced or alimentary thyrotoxicosis). It seemed a little peculiar to us that a patient having had three operations for removal of thyroid tissue (the third time no thyroid tissue having been found) and later x-ray treatment of the neck, should have hyperfunctioning thyroid tissue in her neck. It was, therefore, suggested that the patient was for some reason or other taking thyroid, which was responsible for her symptoms of hyperthyroidism. The following diagnostic procedure was employed:

1. Serum protein-bound iodine was determined and found to be 9.6 micrograms per cent. When compared to the normal value of 3.5 to 7, this elevated value is definitely in the range found in thyrotoxicosis.

2. A tracer dose of radioactive iodine was administered orally. The urine was collected for forty-eight hours. After that time her whole body was scanned with an external gamma-ray Geiger-Mueller counter. No radioactivity could be demonstrated in the neck, over the ovaries, or over any other part of the body.

3. The amount of radioactive iodine excreted in the urine was then determined:

0–24 hours	94.4 per cent
24–48 hours	3.8 per cent
Total in 48 hours	98.2 per cent

The almost total excretion of the radioactive iodine demonstrated that this patient did not have any functioning thyroid tissue in her body. This finding made us suspect further that the patient produced her thyrotoxic state by taking some thyroid substance. On examination of her belongings some pills were found which the patient claimed were luminal tablets given to her by her doctor. Analysis of the pills, however, demonstrated that they contained iodine. In order to test their thyroid activity they were given to an untreated patient having postoperative myxedema. Administration of 2 pills daily for a week produced a rise in BMR from minus 24 per cent to minus 13 per cent and a weight loss of 1.9 Kg. without obvious change in food or fluid intake. This test patient also experienced aching pains in her legs and hot flashes. We believed that this was proof enough to substantiate the fact that the pills were thyroid tablets.

All forms of medication were then made inaccessible to the patient. The BMR, which on admission here was plus 51 per cent, fell in less than three

weeks to normal (0 and plus 3 per cent). The protein-bound iodine fell at the same time from 9.6 micrograms per cent to 1.6 micrograms per cent, the latter value being in the range seen in hypothyroidism.

The patient was interviewed by a psychiatrist but the motivation for self-medication was not found in this single interview, nor would she admit ever having taken thyroid in excess. She was then discharged from the hospital to her local doctor.

Case No. 349932. The second patient, L. R., was a 40-year-old married woman who was referred to the Massachusetts General Hospital for study of symptoms of thyrotoxicosis.

She had been healthy until about 1935. At that time she started to reduce her weight, which was 63.5 Kg., by diet and thyroid medication. In two years she lost about 11 Kg. of weight but felt well. In the summer of 1937 she began to have series of carbuncles and boils. In October, 1938 she was hospitalized elsewhere for treatment of this condition and because of a rather sudden loss of about 5 Kg. in weight. She also complained of increased nervousness and perspiration. Her BMR was plus 49 per cent, pulse rate about 120, and blood pressure 170/90. There was no enlargement of the thyroid and no exophthalmos. The doctor who examined her felt that she presented a clinical picture of thyrotoxicosis. For a year she was treated with iodine and prolonged bed rest and received x-ray therapy to her thyroid. She continued to lose weight, decreasing to 45.5 Kg., despite a high caloric diet. Her BMR was constantly elevated, being between plus 30 and plus 60 per cent. When she did not respond to this treatment it was felt that surgical intervention was indicated and a subtotal thyroidectomy was performed in November, 1939. At operation the thyroid gland was found to be distinctly smaller than normal. A major part of it was removed. The removed gland was reported normal on microscopic examination. At operation, digital and visual search was made for aberrant thyroid gland but none was found. The patient did not improve after operation but continued to feel weak and nervous. She perspired easily and became breathless on climbing stairs. She had attacks of what her physician described as "paroxysmal fibrillations." Her weight remained at about 45 Kg.

In December, 1940 she visited another hospital where further surgery was advised but the patient refused. (The history so far is based upon correspondence with her personal physician).

She was admitted to the Massachusetts General Hospital for study on April 7, 1942. Physical examination revealed a thin, pale woman. Her weight was 38.5 Kg. Her skin was warm and moist. Brown pigmentation was present on both eyelids, and there were also dark brown, macular, circular spots (2 to 3 mm. in diameter) scattered over the cheeks and chin, the forearms and backs of the hands and around the ankles. No pigmentation was present on the palms or on the mucous membranes. There was no palpable thyroid tissue in the neck and no exophthalmos or other eye signs. A coarse tremor of the fingers was demonstrated. Her heart was of normal size, her pulse rate was 90 to 100 and her blood pressure was 130/70. An electrocardiogram was normal. BMR's were plus 31, plus 38 and plus 41 per cent. Protein-bound plasma iodine, determined by Dr. W. T. Salter, was 20.6 micrograms per cent. This value was in the range found in thyrotoxicosis, the normal being 4 to 8 micrograms per cent, according to the method used at that time. Blood cholesterol was 172 mg. per cent. X-ray examination of the chest and neck did not show any substernal goiter, or tracheal compression.

The patient presented a clinical picture of thyrotoxicosis, and the elevated serum

protein-bound iodine also favored this diagnosis, but no thyroid tissue could be felt in the neck. The possibility of a hyperfunctioning ovarian struma was considered but no evidence of ovarian tumor could be found. Therefore, the patient was discharged without a definite diagnosis on April 14, 1942.

She was admitted again to the Massachusetts General Hospital on January 1, 1947, with a diagnosis of Graves' disease. At that time her intervening history disclosed that following discharge in 1942 the patient continued very much as before. In 1944 thiouracil treatment was begun with a dose of 0.2–0.3 Gm. daily, gradually increasing to 0.6–0.7 Gm. daily after three to four weeks, and decreasing to 0.3–0.4 Gm. daily after two to three months. This course lasted for one year and was discontinued when it became apparent she received no benefit. Prior to and subsequent to this thiouracil treatment she took iodine without benefit. She continued to have exertional dyspnea and again complained of nervousness, weakness, easy fatiguability, sweating and heat intolerance. Her appetite remained good and her weight remained stationary during this period until 1946, when she gained 2 Kg.

Throughout the period following her first admission the one symptom which had progressed was the frequency of her episodes of paroxysmal rapid arrhythmia. Occurring occasionally at first, they became later more frequent, and were almost constant for the three months prior to admission. No beneficial effect was noted from quinidine or digitalis. She also took 0.6 Gm. of thiouracil a day, for the three months before admission—again without effect. Her nervousness had increased. She had not taken iodine, nor had she had a cholecystogram during the preceding year.

The examination showed a thin, pale woman weighing 47 Kg. She had cool, dry skin which was pigmented, as in 1942. There was no pigmentation in the mouth. She had a marked tremor of the fingers but no eye signs. No goiter could be felt and the presence of thyroid tissue was questionable. Her heart was slightly enlarged. Her pulse rate was 100 to 130 and her blood pressure was 140/95. An electrocardiogram showed auricular fibrillation with a ventricular rate of about 140. The blood-sedimentation rate was 5 mm. per hour. Cholesterol was 167 mg. per cent. Her BMR's were plus 36 and plus 30 per cent. The protein-bound iodine of the serum was 14.5 micrograms per cent (normal, 3.5 to 7.0 micrograms per cent).

On the whole, the patient presented the same clinical picture as she had on the first admission in 1942, the only apparent change being onset of fibrillation. She had a long history suggesting thyrotoxicosis and definite symptoms of hyperthyroidism including an elevated basal metabolic rate and definitely elevated serum protein-bound iodine. As before, the main problem was to determine whether or not this patient had endogenous thyrotoxicosis.

In her history there were several peculiar features. The patient did not improve at all following the subtotal thyroidectomy in 1939. The thyroid gland was at that time found to be grossly smaller than normal and microscopically normal. She did not respond to one year's treatment with thiouracil. She did not present a characteristic picture of thyrotoxicosis. There were no eye signs. In the period between the two Massachusetts General Hospital admissions she developed auricular fibrillation. It was of the type that appears in thyrotoxicosis. It was paroxysmal in nature, responded

poorly to quinidine and digitalis and appeared in a relatively young woman without other obvious cause. Mitral stenosis was considered but no characteristic murmurs could be heard even when the cardiac rhythm was normal. Active rheumatic fever was considered as a faint possibility, but the blood-sedimentation rate was found to be normal. A diagnosis of rheumatic fever could explain neither her high BMR nor the long clinical course.

In this situation it was decided to give the patient a tracer dose of radioactive iodine. The radioactive iodine excretion test gave the following result:

Time	*Urine volume*	*Per cent of administered I^{131} excreted in urine*
0–24 hours	680 ml.	53.2
24–48 hours	320 ml.	2.8
Total in 48 hours:		56.0

This result was in the range considered normal. Since no thyroid tissue could be felt in the neck a careful search for radioactivity over the whole body was made with an external gamma-ray Geiger-Mueller counter. This revealed no activity in the neck, over the ovaries or in any other part of the body. The results of the direct measurements therefore did not check with the urinary excretion figures. We should have been able to demonstrate some radioactivity in the body if she had excreted only 56 per cent of the dose. It was noted, however, that the urinary volumes were very small, being 680 cc. and 320 cc., and we thought that perhaps some of the urine was discarded by mistake. Both the nurses and the patient denied this possibility. We therefore decided to repeat the radioactive iodine excretion test and to collect the urine by an inlying catheter. The patient firmly objected to such a procedure but was finally persuaded to submit to it. During the test the patient asked several times to have the catheter removed because she said she had to make "important" telephone calls. However, this was not permitted. The result of the second test showed the following:

Time	*Urine Volume*	*Per cent of administered I^{131} excreted in urine*
0–24 hours	1090 ml.	85.7
24–48 hours	900 ml.	11.6
Total in 48 hours:		97.3

In this second test the urine volumes were almost double those of the first test. It is very likely that the patient herself had intentionally discarded some urine during the first test. We know by experience that the urine volume alone does not play a role in the excretion of the radioactive iodine. Since this second test revealed that the patient was excreting almost all the radioactive iodine administered, the possibility that this patient's symptoms were caused by hyperfunctioning thyroid tissue was excluded.

We were then confronted with the following contradictory evidence: 1. The patient's history and clinical picture seemed to indicate hyperthyroidism. 2. Her serum protein-bound iodine was elevated to the range found in thyrotoxicosis. 3. Her radioactive iodine excretion was almost 100 per cent. It seemed to us that the most feasible explanation of the situation was that the patient was taking thyroid. However, when this possibility was discussed with her she categorically denied it, became very upset, and immediately decided to leave the hospital. Our plans to have her seen by a psychiatrist could therefore not be carried out. The diagnosis of self-induced thyrotoxicosis is not fully proved in this patient, but there is little doubt in our minds that this must have been the essential condition.

DISCUSSION

Typically, in thyrotoxicosis one finds an elevated serum protein-bound iodine and a low urinary excretion of tracer doses of radioactive iodine. In the two cases reported here, however, an elevated serum protein-bound iodine was associated with a *high* urinary excretion of radioactive iodine, a combination which apparently is characteristic of alimentary hyperthyroidism. The serum protein-bound iodine may usually be accepted as an index of circulating thyroid hormone. It is, of course, impossible to differentiate between the thyroid hormone produced by the individual's own gland and thyroid hormone derived from medication. However, the radioactive iodine tolerance test is of assistance in determining the origin of a high value of "hormonal" iodine. If the elevated protein-bound iodine is caused by an increase in activity of the patient's thyroid gland then this gland's avidity for iodine is also increased and this can be demonstrated either directly by *in vivo* measurements of radioactivity over the gland or indirectly by a low urinary excretion of the radioactive iodine. If, on the other hand, the high serum protein-bound iodine is caused by prolonged administration of thyroid, then the gland has a decreased avidity for iodine and the radioactive iodine test gives a high excretion value.

It might be argued that the two patients whose cases are reported here probably had little or no thyroid tissue left and therefore would be expected to excrete practically all of the administered radioactive iodine. However, even in a patient with an intact thyroid gland, the same pattern

of elevated serum protein-bound iodine and high excretion of radioactive iodine may be produced by excessive ingestion of thyroid, as illustrated by the following case:

A.P. was a 35-year-old married woman who was healthy until she reached puberty at the age of 14. Shortly after this she began to have attacks of somnolence, and a diagnosis of narcolepsy was made by an outside physician. At that time she was found to have a low basal metabolic rate of from minus 25 to minus 32 per cent. For the past five years her physician had prescribed fairly large doses of thyroid; for the first three years she took 0.3 Gm. daily and for the last two years, 0.4 to 0.5 Gm. daily. She developed palpitation and dyspnea on exertion, but denied nervousness or intolerance to heat. Examination revealed a jumpy and nervous woman. Her skin was of normal temperature but somewhat moist and there was a fine tremor of the extended fingers. Her thyroid was palpable but not enlarged. No eye signs were present. Her heart was of normal size. Her pulse rate was 86. Her blood pressure was 130/70. The basal metabolic rate was plus 3 per cent. Her serum protein-bound iodine was 9.4 micrograms per cent and she excreted 86.7 per cent of a tracer dose of radioactive iodine in forty-eight hours.

After these studies were completed the thyroid medication was stopped. Eight weeks later the patient had gained 6 Kg. in weight. She was much more quiet than she had been at the first examination. The tremor of her fingers had disappeared. Her pulse rate had fallen to 68. At this time the laboratory studies were repeated. Her basal metabolic rate was minus 17 per cent. Her serum protein-bound iodine was 5.4 micrograms per cent and the radioactive iodine excretion test showed 66.8 per cent excreted in forty-eight hours.

When this case was first studied we found the combination of elevated serum protein-bound iodine and high excretion of radioactive iodine which we had found in the two other cases. When the thyroid medication was stopped, both the serum protein-bound iodine and the excretion of radioactive iodine decreased to values definitely in the normal range. The result of the radioactive iodine excretion test suggests that the thyroid medication depressed the function of the thyroid gland, as would be expected from previous observations (Farquharson and Squires (11); Riggs, Man and Winkler (6)).

Unfortunately, the combination of an elevated serum protein-bound iodine and high urinary excretion of radioactive iodine, while characteristic of, is not by itself pathognomonic of, alimentary thyrotoxicosis. In a patient with endogenous thyrotoxicosis treated with either thiouracil or iodide one may find the same laboratory pattern until the disease is brought under control. The radioactive iodine excretion, which in the untreated thyrotoxic patient is low, becomes high when thiouracil treatment is used because this drug prevents the utilization of iodide by the thyroid gland. Therefore, when a tracer dose is administered, most of it will be excreted in the urine. When iodide is given therapeutically, the concentration of inorganic iodide in the blood is so great compared to the amount of iodide administered in a tracer dose, that differences in the thyroid's avidity for

iodide will not be demonstrated. Furthermore, the gland becomes saturated with iodide and will collect very little more.

A patient who has recently had a cholecystogram may also show the same pattern of laboratory data. The organically bound iodine in the gall-bladder dye may keep the serum protein-bound iodine elevated for a long period of time, perhaps as long as a year (12). It will also increase the excretion of radioactive iodine. As an example, we recently studied a patient with a classical picture of Graves' disease. Untreated, the patient excreted only 12.6 per cent of a tracer dose of radioactive iodine. She then had a cholecystogram. Two days later the radioactive iodine test was repeated. In the second test the patient excreted 79.5 per cent. We do not know how long this effect on the radioactive iodine excretion lasts nor the mechanism by which it works. Experiments to elucidate these problems are in progress in our laboratory. We believe, however, that such things as previous cholecystograms might be responsible for the fact that thyrotoxic patients occasionally have a very high excretion of radioactive iodine.

SUMMARY

A case of secretly self-induced thyrotoxicosis (thyrotoxicosis factitia) is described, and a second case in which circumstantial evidence strongly favored the same diagnosis is also discussed. In both patients the clinical picture was indistinguishable from that of endogenous thyrotoxicosis. The differential diagnosis was facilitated by the use of tracer doses of radioactive iodine. In endogenous thyrotoxicosis the urinary excretion of radioactive iodine is low compared to that of euthyroid individuals. In thyrotoxicosis factitia the excretion of radioactive iodine is higher than normal. Since the serum protein-bound iodine is elevated independently of the source of iodine-containing hormone, this test is not contributory to the differential diagnosis. Other conditions which may produce the same combination of elevated serum protein-bound iodine and high excretion of radioactive iodine are discussed.

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HORMONAL FACTORS INVOLVED IN THE REGULATION OF BASAL BODY TEMPERATURE DURING THE MENSTRUAL CYCLE AND PREGNANCY*

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THE biphasic aspect of the basal body temperature during the normal menstrual cycle is now a well-established fact. The temperature rise of three-fifths to one degree Fahrenheit which occurs at or about the time of ovulation is a phenomenon frequently used diagnostically and therapeutically. There are frequent variations from this typical biphasic curve, with its abrupt midcycle rise, which have been commented upon elsewhere (1), but generally speaking the rise maintains itself until the onset of the next menstrual period during which it drops to its preovulatory level. In the event that pregnancy occurs and the next menstrual period fails to appear when expected, the basal temperature does not return to its preovulatory level, but continues at or about the level maintained during the latter two weeks of the menstrual cycle. This, in itself, is a fairly accurate diagnostic sign of pregnancy.

The purpose of this paper is two-fold: first, to present evidence as to the actual cause of the temperature rise during the latter half of the menstrual cycle and second, to investigate the progress of the basal body temperature throughout pregnancy.

METHODS AND OBSERVATIONS

In order to ascertain the effect of steroid ovarian hormones on basal body temperature, six patients with secondary amenorrhea and with little or no endometrial activity as determined by biopsy, were studied in the following manner. After a temperature base line had been established, the patients were given by mouth a priming dose of estrogen consisting of 3 to 5 mg. of either estrone sulfate[1] or diethylstilbestrol daily for about two weeks. Various amounts of progesterone, ranging from 10 to 25 mg. daily,

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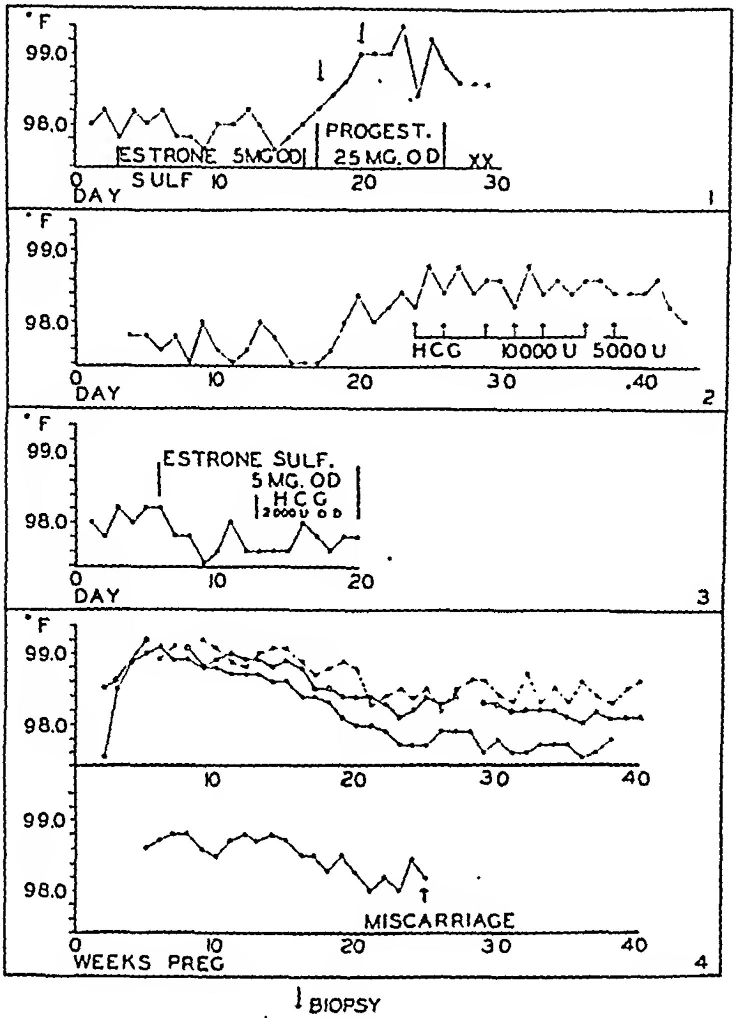
[1] The estrone sulfate (Premarin) and chorionic gonadotropin (APL) were supplied to us through the courtesy of Dr. Murray Scott of Ayerst, McKenna and Harrison; the progesterone (Proluton), through the courtesy of Dr. Edward Henderson of the Schering Corporation.

were then administered for from seven to fourteen days. During this time the estrogen was either discontinued or maintained at half the original dosage. The temperature curves obtained from these patients during treatment indicate that estrogen may produce a slight depression of the basal temperature whereas progesterone, either with or without estrogen, produces a very definite rise. In some instances the latter amounted to a degree or more (Fig. 1). These observations are in substantial agreement with the findings of Barton and Weisner (2). Frequent biopsies to ascertain the effect of these substances on the endometrium confirmed the activity of the administered hormones. Characteristic endometrial responses are demonstrated in Figures 5 and 6.

In addition to this effect of ovarian steroids on the temperature levels of amenorrheic patients, it has been shown previously that the administration of large amounts of chorionic gonadotropin to normal women during the latter half of the menstrual cycle maintains the postovulatory rise and prevents menstruation for a considerable length of time (3). This experiment has been repeated by one of the authors (4) with confirmation of these findings (Fig. 2). It has been assumed that the chorionic gonadotropin stimulates the existing corpus luteum to produce progesterone and that the latter substance is responsible for the maintenance of the temperature rise.

In this connection there is also the possibility that the chorionic gonadotropin itself is directly responsible for the maintenance of the temperature rise. To test this alternative, a 31-year-old bilaterally ovariectomized woman was given an equivalent amount of chorionic gonadotropin after priming with estrogen. No rise in temperature occurred (Fig. 3). It therefore seems probable that the maintenance of the temperature rise during treatment in the normal woman is due to the action of progesterone secreted by the ovary in response to the injected gonadotropin.

In view of these findings one would expect that the elevated basal body temperature would be maintained throughout pregnancy, since progesterone is present in gradually increasing quantities, at least as determined by measurement of the pregnandiol excretion (5). To test this hypothesis, three patients were instructed to continue temperature charts throughout the period of gestation. One woman kept records during two pregnancies, the second of which terminated in a five and one-half months miscarriage due to unknown causes. It is interesting and surprising to note that in each case the temperature in the fourth month of pregnancy began a gradual drop which reached preovulatory levels by the beginning of the fifth month (Fig. 4). Since these observations were made, two similar charts have been included in a report concerning the time of ovulation and conception (6).



FIGS. 1–4

1. Temperature chart of patient treated with estrone sulfate, 5 mg. daily, followed by progesterone, 25 mg. daily. Arrows indicate dates of endometrial biopsy. XX indicates onset of bleeding.

2. Temperature chart of a normally-ovulating patient treated with human chorionic gonadotropin following the ovulatory temperature rise.

3. Temperature chart of a bilaterally ovariectomized woman treated with daily doses of human chorionic gonadotropin following previous treatment with estrone sulfate.

4. Temperature charts of 3 patients followed throughout pregnancy, the base line representing the number of weeks since the last menstrual period. One patient recorded her temperature throughout a second pregnancy, which terminated in a miscarriage at 25 weeks.

DISCUSSION

It is apparent from the experimental data presented above that the elevation of basal body temperature in hormone-treated amenorrheic women is due to the action of progesterone. That this is also the case in normal women during the latter half of the menstrual cycle and early

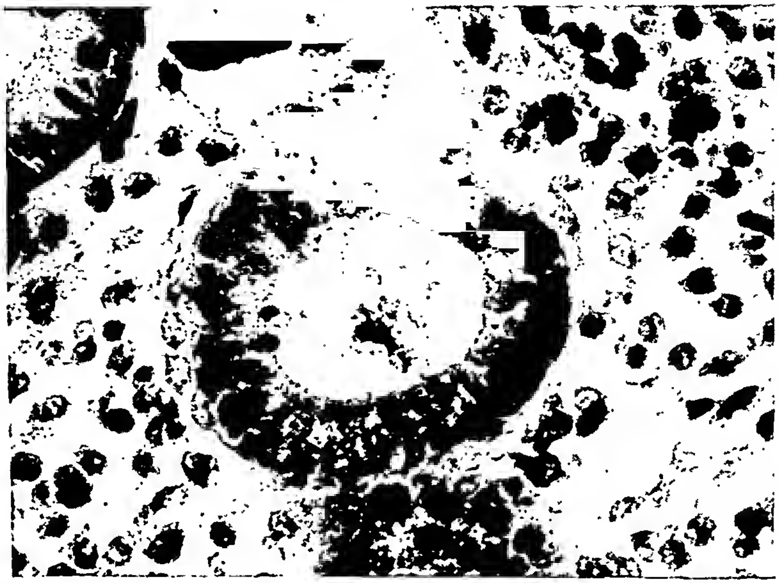


Fig. 5. Photomicrograph of an endometrial gland from the first biopsy indicated in Figure 1, to demonstrate the response to the estrogen treatment.

Fig. 6. Photomicrograph of endometrial glands from the second biopsy indicated in Figure 1, to demonstrate the response to the progesterone treatment.

pregnancy is strongly suggested by the close temporal correlation between the rise and decline of basal temperature and luteal activity. On the other hand, if this is the case, it is surprising that a temperature drop should occur during midpregnancy at a time when pregnandiol excretion studies indicate a constantly rising level of circulating progesterone.

Several possibilities exist which might account for the above discrepancy. For instance, it is possible that a refractory state develops in connection with the thermogenic action of progesterone or that the action of progesterone is overcome by stronger thermodepressant factors. Lack of data in this regard precludes further speculation. A second alternative is that the maintenance of the temperature rise during early pregnancy may be due, at least in part, to unknown factors other than progesterone. Here again the paucity of information makes the identification of any such factors a matter of conjecture. It is interesting to note, however, that Caffier (7) has reported that trophoblast grown in tissue culture possesses proteolytic activity which disappears about the fourth month of gestation. Could it be that the lytic activity of the trophoblast on the decidual tissue of the mother during the early months of pregnancy results in a slight febrile response on the part of the latter? Certainly the time relationship is interesting and since widespread lysis of tissue often produces a febrile response, this possibility may have some factual significance.

An additional explanation with important implications is that the decline in basal body temperature during pregnancy, as in the menstrual cycle, may reflect a decrease in physiologically active progesterone. This concept is at variance with the generally accepted belief that the maintenance of gestation is dependent upon increasingly high levels of progesterone activity. However, Atkinson and Hooker (8) have recently pointed out that the prevalent view is open to question. Urinary excretion of pregnandiol in woman is accepted as evidence of the presence of circulating progesterone. Unfortunately, however, few or no direct data are available relating the levels of circulating progesterone to the urinary output. Furthermore, experimental studies in lower animals indicate that the level of physiologically active progesterone may decrease considerably during the second half of gestation (8, 9, 10). The decline of the elevated basal temperature at midpregnancy in the human is consistent with a hypothetical decline of active circulating progesterone. Further work is indicated before the question can be settled conclusively.

SUMMARY AND CONCLUSIONS

In order to ascertain the cause of the well known postovulatory rise in basal body temperature in normal cyclic women, six patients with amenorrhea and with little or no endometrial activity were given sequential ther-

apy of estrogen followed by progesterone. The patients were followed by temperature charts and endometrial biopsy. The basal body temperature invariably rose significantly (three-fifths to one degree Fahrenheit) during progesterone therapy.

The postovulatory temperature rise was also maintained and menstruation postponed ten to thirty days in normal women by the administration of chorionic gonadotropin. However, chorionic gonadotropin administered to a castrate following estrogen priming did not result in any significant change in basal temperature. It was assumed, therefore, that the maintenance of the postovulatory temperature rise by chorionic gonadotropin was due to its luteotropic effect and that here also progesterone produced the temperature response.

To determine whether the rise were maintained throughout gestation, three women (one through two pregnancies) kept temperature charts until delivery. The temperature fell to its preovulatory level uniformly between the fourth and fifth month in all cases. This was in spite of the commonly supposed steady rise in progesterone activity as determined by pregnandiol excretion. Some conjecture, but no final explanation is offered for this interesting phenomenon.

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THE CAUSE OF PHYSIOLOGIC BASAL TEMPERATURE CHANGES IN WOMEN*

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THE clinical application of basal temperature changes in the study of ovarian function has met with wide acceptance since the publications of Tompkins (1), Davis (2) and others (3, 4, 5). This simple physiologic phenomenon has provided a much-needed tool for the interpretation of normal and abnormal ovarian activity. The mechanism for these temperature fluctuations has not been elucidated. The following observations were carried out in an attempt to throw additional light on this subject.

In the past three years well over 1000 temperature graphs have accumulated in the study of sterility and other endocrine disorders, as well as in a large group of normal individuals who were used as controls. These graphs have been prepared by the patients themselves after careful instructions on how to keep a basal temperature record. Oral temperatures have been used throughout, since it has been demonstrated that the pattern is similar to rectal temperatures and that the latter provide no greater degree of accuracy. Most patients have obtained readings over a period of three or four months but many have kept a continuous graph for a year or longer.

A careful study of these graphs reveals some interesting facts which are worthy of comment. About 75 per cent of patients present graphs in which the temperature curve is sufficiently typical to provide valid data concerning ovarian function. The remaining 25 per cent of patients have such violent temperature fluctuations that the pattern may be completely disturbed. These are highly strung, nervous individuals, many of whom lead irregular lives, working, eating, sleeping and worrying to such an extent that these emotional, physical and metabolic stimuli mask the hormonal control of the body basal temperatures. Furthermore, in the interpretation of temperature graphs isolated irregularities in a curve must not be given undue importance because the general pattern extending over one or more complete cycles is a better index of ovarian activity. In spite of these handicaps, a temperature graph carefully prepared by a patient is of inestimable value.

There has been considerable discussion as to the interpretation of the

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ovulatory rise in temperature in the light of its great practical significance. Clinically, as well as for academic reasons, it is important to know the exact time of ovulation. The life of the human ovum is very short, certainly less than twenty-four hours. Spermatozoa survive a longer period but their fertilizing power is definitely limited. Problems of human reproduction would be simplified if we could indicate the exact time of ovulation.

In some graphs a temperature drop can be seen just before the ovulatory rise, usually the day before. Some authors have interpreted this preovulatory drop as the time of ovulation. Coitus during this period has resulted in some pregnancies. However, the drop is not a constant phenomenon and is difficult to interpret. In the graphs we studied, a typical drop prior to the ovulatory rise was evident in less than fifty per cent. Furthermore, this drop in temperature could be ascertained in many cases only after the ovulatory rise occurred. Thus, its clinical value is lost because of the inability of the patient to interpret the graph correctly.

There is considerable evidence that ovulation may occur during the rise in temperature. The best evidence to support this view has been presented recently by Greulich (6). He examined ovaries of women removed at the low point in the basal temperature graph. He found large follicles in the ovaries but no recently ruptured corpora lutea. In a second group of women he examined the ovaries at laparotomy after the temperature had passed the low point and had begun to rise and he found new corpora lutea. He concluded that ovulation occurs after the temperature has passed its low point.

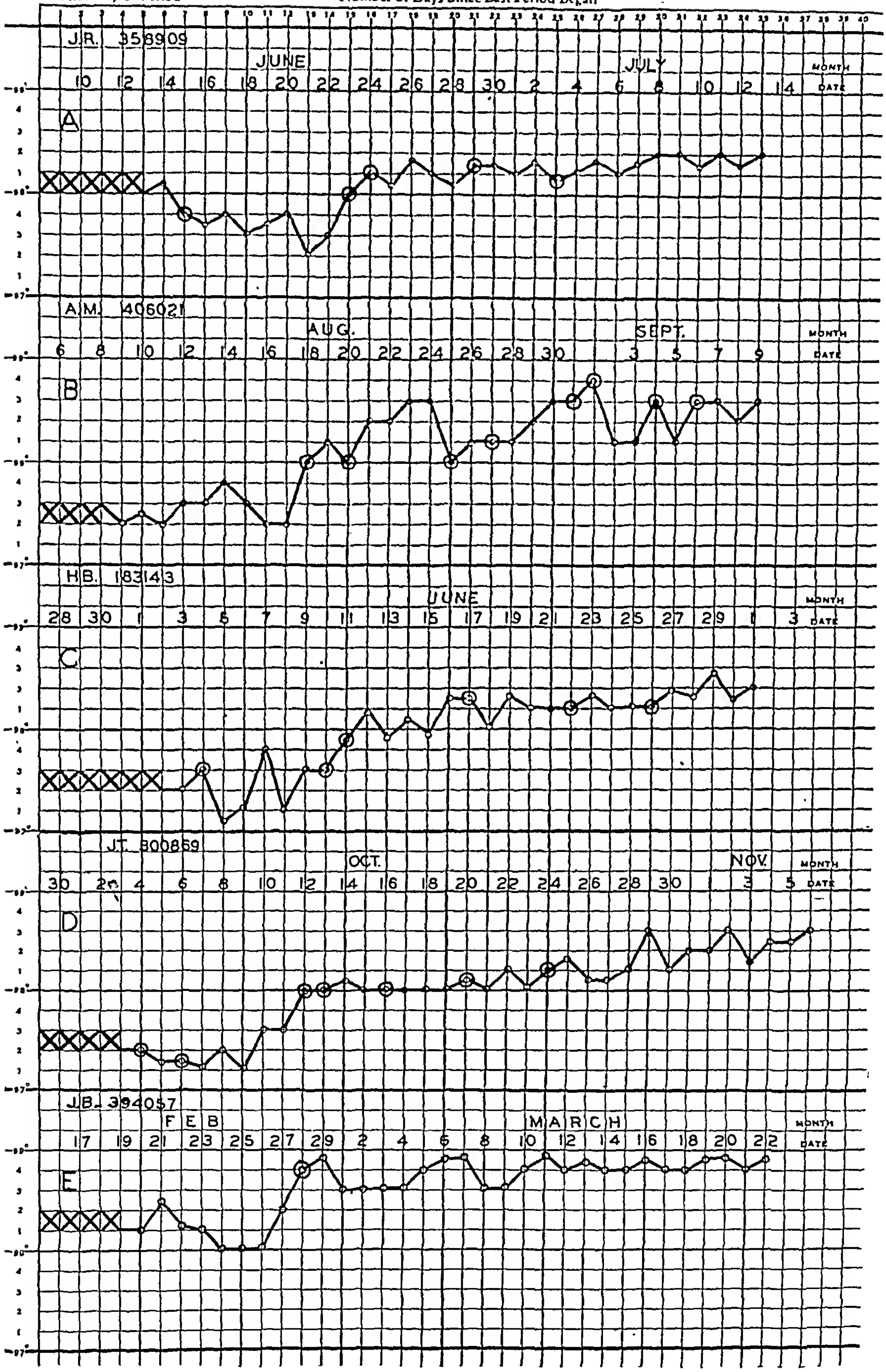
The present study comprises three parts: 1) the time of ovulation in relation to body basal temperature changes; 2) the artificial reproduction of the temperature curve in women with primary amenorrhea and following castration, by means of estrogens and progesterone; and 3) the study of ovarian activity in the absence of the uterus.

DATA

In a study of 100 basal temperature graphs of women who conceived, we found 24 records in which coitus took place during the period when the temperature had passed the low point and was rising to the postovulatory level. Five examples are shown in Figure 1. These women had regular cycles prior to conception, recurring every twenty-seven to thirty-one days. In an additional 48 patients, coitus took place at frequent intervals extending from the low point prior to the rise in temperature until the rise reached its peak.

It is generally assumed that in women who have regular cycles varying from twenty-eight to thirty-one days, ovulation is most likely to occur during the interval between days 10 and 18. Data collected by Hartman

First Day of Period Number of Days Since Last Period Began



(7) in the macacque, as well as records of isolated coitus in women confirm this observation. Furthermore, the life of the human corpus luteum has been estimated to be fourteen or fifteen days. In Figure 1A, coitus occurred on days 7, 15, 16 and 21. It is unlikely that the fruitful coitus took place on day 7 or 21. Thus there is good presumptive evidence that ovulation and pregnancy occurred on day 15 or 16 during the rise in the temperature curve, rather than on day 13, the low point in the curve preceding the rise. In Figure 1B, coitus occurred on days 13 and 15 and not again until day 20. It is fair to presume that fertilization occurred during one of these days and during the temperature rise. Similar deductions have been drawn from data in Figures 1C and 1D. Finally, Figure 1E shows a graph from a patient who had regular menstrual cycles recurring every twenty-eight to thirty-one days. She had not conceived because of a severe oligospermia in the husband. For this reason the frequency of coitus was reduced to once or twice a month. Thus coitus took place only once, on day 13, and it was followed by conception. This coincided with the peak of the luteal rise in basal temperatures.

The present study was not contemplated at the time the patients prepared their graphs. The women were primarily patients who complained of infertility and a basal temperature record was a part of the investigation and treatment of their failure to conceive.

There is considerable evidence that the high level of the temperature during the luteal phase of the cycle is brought about by progesterone. The

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FIG. 1. Basal temperature graphs in a study of sterility in patients who ultimately conceived, showing the fertile cycle in each case.

(A) J. R. 358909. This patient had fairly regular 29 to 31-day cycles as indicated in her temperature graph. Coitus, marked by a circle, occurred on days 7, 15, 16 and 21. She probably conceived on day 15 or 16 as the temperature was reaching the top of the rise.

(B) A. M. 406201. This patient had regular 27 to 28-day cycles. Coitus occurred on days 13 and 15 and not again until day 20. She most likely conceived on day 13 or 15 after the temperature had passed its low point on day 12.

(C) H. B. 183143. The ovarian cycles were fairly regular, varying from 28 to 31 days. The low point in the temperature curve occurred on day 12: however, coitus took place on days 8, 14, 15 and 21. She probably conceived on day 14 or 15.

(D) J. T. 300869. This patient had regular 27 to 29-day cycles. The low point in the temperature curve was on day 10. Coitus occurred on days 5, 7, 13, 14, 17 and 21. It is more than likely that pregnancy occurred on days 13 or 14.

(E) J. B. 394057. This patient had regular cycles recurring every 28 to 31 days. Her husband had a pronounced oligospermia. Coitus occurred only once, on day 13 in the cycle illustrated, following which she conceived. Note that this was near the peak of the ovulatory rise.

following facts support this view. The temperature remains at its elevated level during the active secretory life of the corpus luteum. We have been able to correlate the output of pregnandiol and the elevated temperature. The rise in temperature is followed by an increased output of pregnandiol which persists throughout the luteal phase. Twenty-four to thirty-six hours prior to menstruation the corpus luteum ceases to be active, pregnandiol

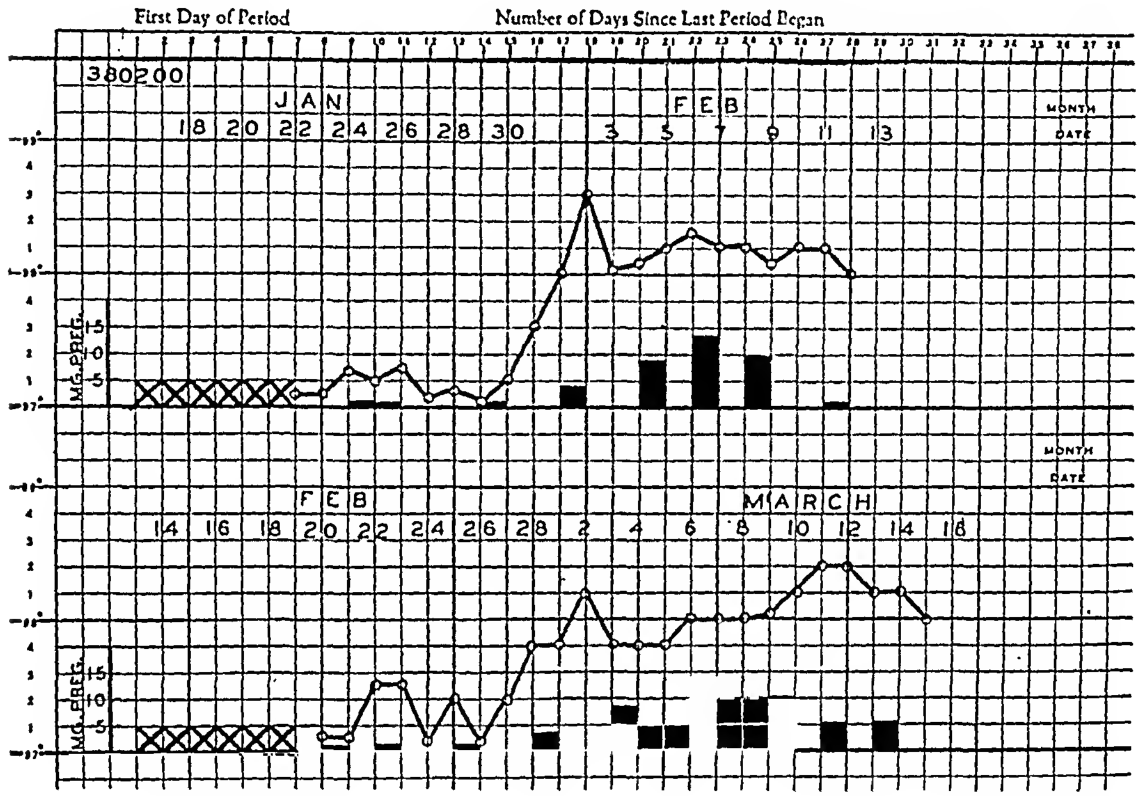

FIG. 2. Two menstrual cycles, 28 and 31 days in length, showing the relationship of pregnandiol excretion in the urine (black columns) and the basal temperature fluctuations. There is a good correlation between the excretion of pregnandiol and the luteal rise in the temperature.

levels drop rapidly and the temperature curve slides down to the preovulatory level.

In a small group of young women with regular menstrual cycles who were studied in the clinic, an attempt was made to correlate the temperature graph and the output of pregnandiol in the urine. In Figure 2 it can be seen that very small amounts of pregnandiol, probably extra-ovarian in origin, are recovered prior to the temperature rise. The first appreciable amount of pregnandiol appears after the rise has begun. The daily output increases, reaching its maximum about the middle of the luteal phase. There is no way of correlating ovulation, corpus luteum activity and pregnandiol output more accurately, for there must be a lag between proges-

terone excretion by the corpus luteum and its metabolism and conversion into pregnandiol.

If pregnancy occurs, the temperature fails to drop and continues at the postovulatory level throughout the continued active life of the corpus luteum. Furthermore, the postovulatory rise in temperature persists for approximately fourteen or fifteen days, regardless of the length of the cycle, since this is the approximate life of the corpus luteum as a secretory gland. Lastly, during an anovulatory cycle there is no rise in temperature, and it remains at approximately the same level until bleeding ensues.

The second part of this presentation concerns itself with the artificial production of typical body basal temperature fluctuations by the administration of estrogens and progesterone,[1] in women with no ovarian activity. Two groups of young women were selected: 1) four young women with primary amenorrhea who had been studied in our clinic for many months; and 2) four young women in whom it had been necessary to remove the ovaries, tubes and uterus because of disease of these pelvic organs. Basal temperature readings were charted over a four-month period, and their curves were typical, in that none exhibited any sustained rise and readings fluctuated from day to day as in the male. Pregnandiol determinations were made from 24-hour urine collections at periodic intervals, but little or no pregnandiol was present. It was therefore assumed that these young women had no ovarian activity of their own.

Ethinyl estradiol was administered orally to two patients in each group. They received 0.3 milligram daily throughout this first part of the experiment. About two weeks after the onset of therapy, 10 milligrams of progesterone in oil was given intramuscularly daily for twelve to fifteen days, following which progesterone and estrogen were discontinued. The temperature graphs were similar in the four patients. Examples are shown in Figures 3 and 4.

The estrogenic substance produced no change in the characteristics of the curve prior to medication. Within twenty-four hours after the patient received progesterone the temperature rose abruptly, perhaps more so than in the normal cycle. It remained elevated during the entire period in which the patient received progesterone. However, following the discontinuation of estrogens and progesterone the temperature dropped slowly to the general level existing during the estrogenic phase. The decline was very slow, lasting three to four days, which is much longer than in the natural cycle. In general, the curve of the recovery of pregnandiol from the urine

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[1] We wish to thank Dr. Edward Henderson and the Schering Corporation for liberal supplies of progesterone and estrogens used in this study.

in all of these patients followed the temperature curve, but the amount of pregnandiol recovered varied in each patient.

It can be said that the basal temperature curve of the artificial hormonal

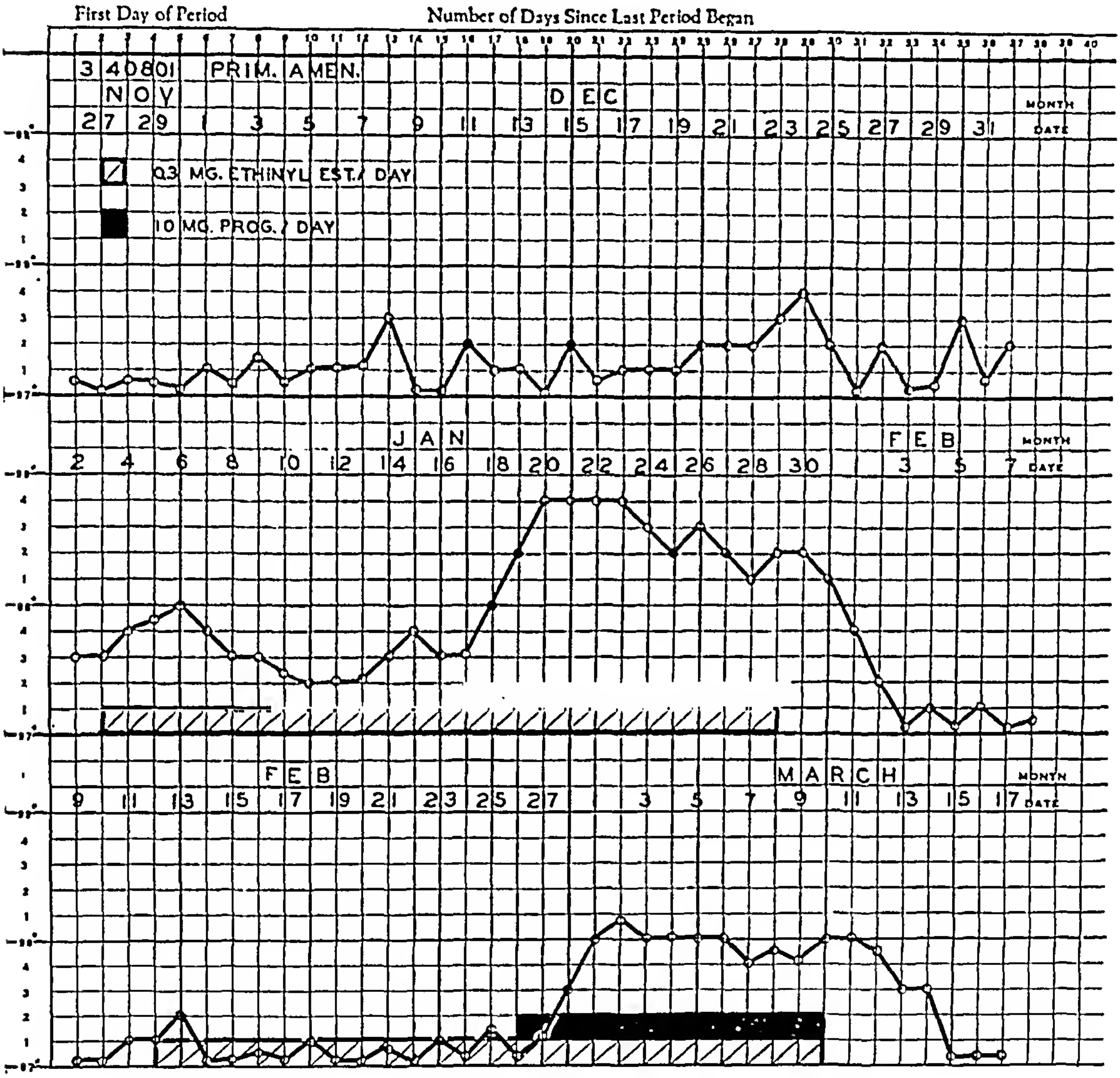

Fig. 3. The basal temperature graph of a patient with primary ovarian failure. Note the irregularity of the curve prior to the oral administration of ethinyl estradiol. During the estrogenic phase the temperature remains low and fairly constant. Following the parenteral administration of progesterone the rise is rather abrupt but after the cessation of medication the decline is more gradual. The artificial hormonal cycle thus induced simulates the natural ovarian cycle. The length of the estrogenic and progesterone phases can be varied at will.

cycle induced in these patients with no ovarian function of their own resembled the natural cycle with two exceptions. The rise was more rapid and the decline was more prolonged in the artificial cycles than in most natural ones. The rapid rise may be due to the sudden intake of a consider-

able amount of progesterone whereas in the normal cycle the developing corpus luteum may liberate progesterone more slowly. It may likewise vary with the rate and amount of secretory activity in different individuals thereby altering the pattern of the luteal phase of the cycle.

As a control for these observations one cubic centimeter of cottonseed oil was substituted for the 10 milligrams of progesterone in one cubic centimeter of oil. The rest of the experiment was not varied. There was no rise in basal temperature, and the pattern of the curve did not change. This

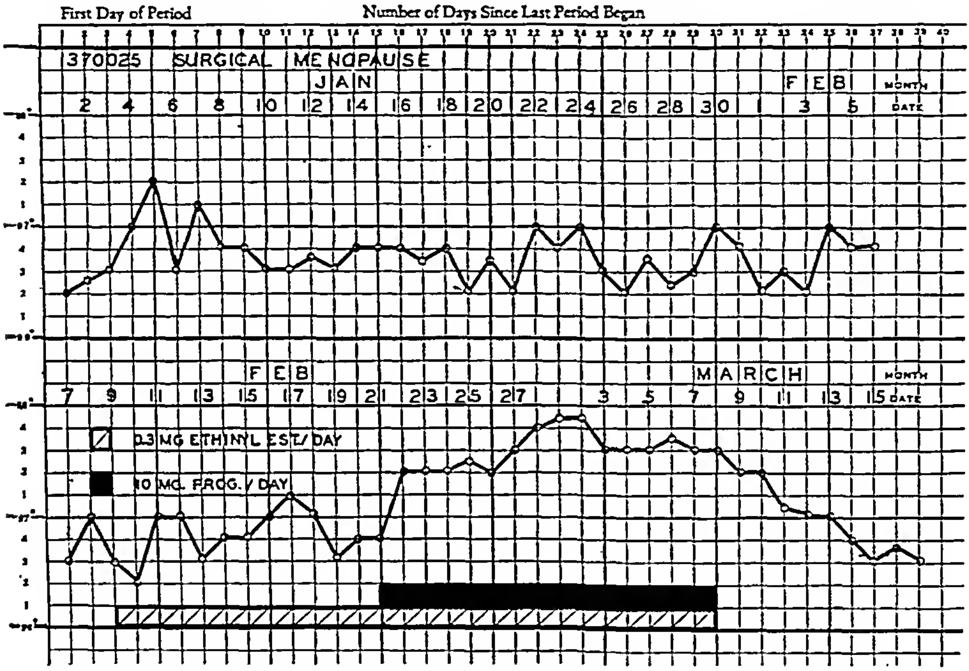

FIG. 4. The basal temperature graph of a 28-year-old patient who had undergone a surgical menopause. Note the low level of the curve prior to medication. The administration of estrogen did not alter this curve. However, progesterone produced a prompt rise and this elevated temperature continued until the cessation of therapy.

removed the parenteral mode of administration of the progesterone as a factor in the elevation of the temperature above the estrogenic level (Fig. 5).

In three of the patients, pregneninolene was substituted for progesterone in oil; otherwise the experiment was not altered. Fifty milligrams of Pranone was administered by mouth daily. The only change in the pattern of the artificial cycle was the slow rise of the basal temperature after the administration of pregneninolene orally, in comparison to the rapid rise following progesterone in oil (Fig. 6).

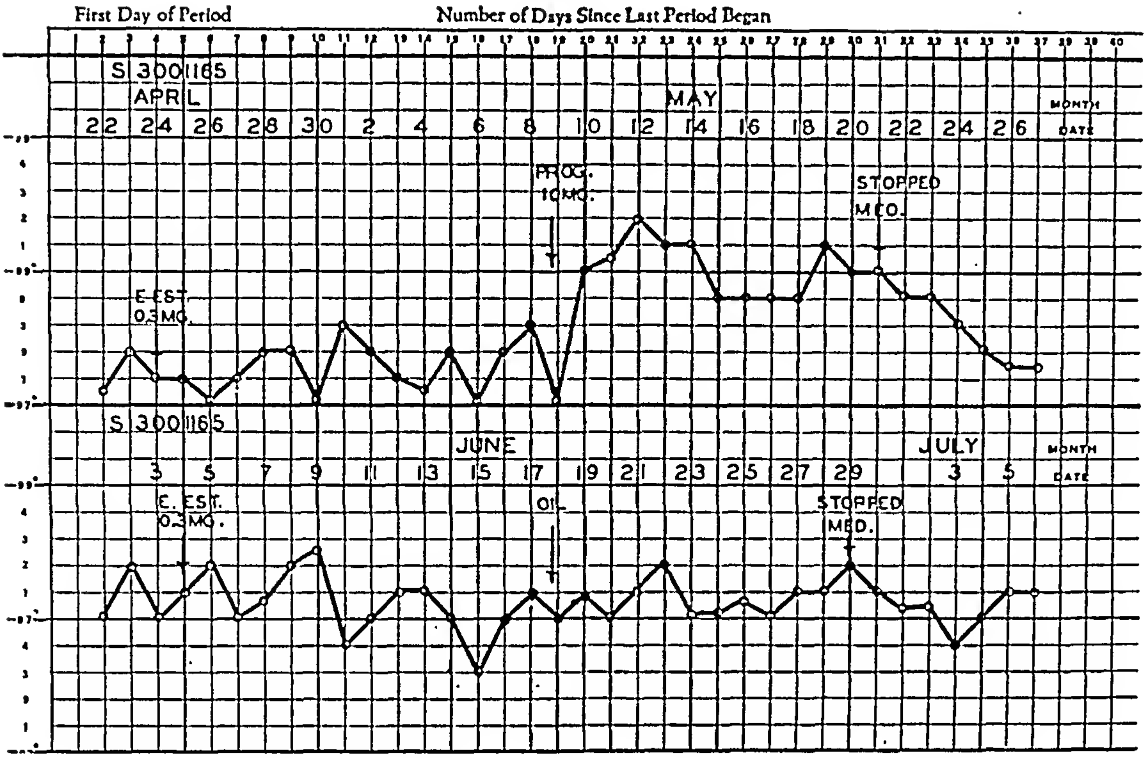

Fig. 5. In this graph an artificial basal temperature cycle was induced by estrogen and progesterone. In a second cycle one cubic centimeter of cottonseed oil was substituted for the progesterone. Note that no rise in temperature followed the substitution of the cottonseed oil, thereby eliminating the factor of parenteral administration in the causation of temperature elevation.

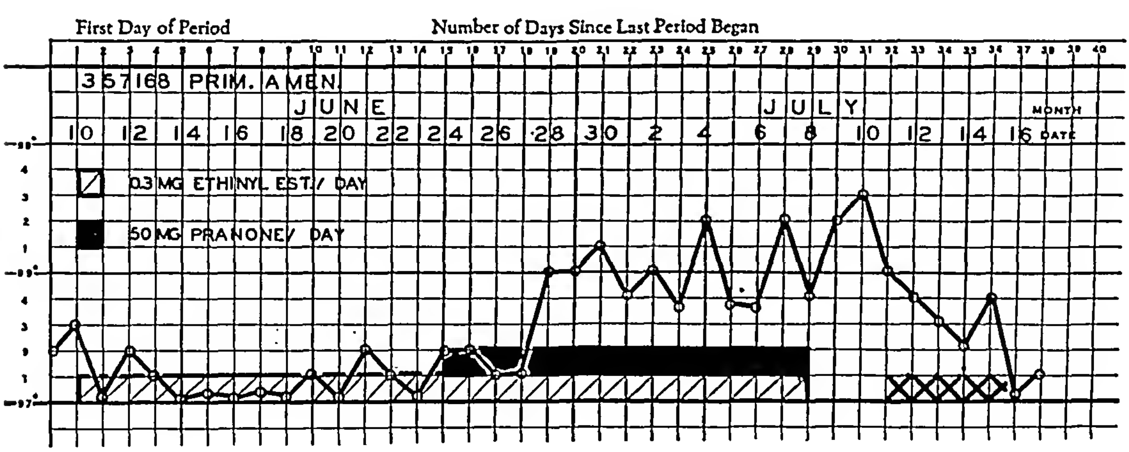

Fig. 6. In this artificial basal temperature cycle pregneninolene, an active oral progestin, was substituted for progesterone in oil. Note the gradual rise in the temperature in contrast to the more abrupt rise following the administration of progesterone parenterally. This patient exhibited typical progesterone withdrawal bleeding.

In a previous observation methyltestosterone was administered to young women with regular cycles who complained of severe dysmenorrhea. Basal temperature graphs have been kept by these women during the last

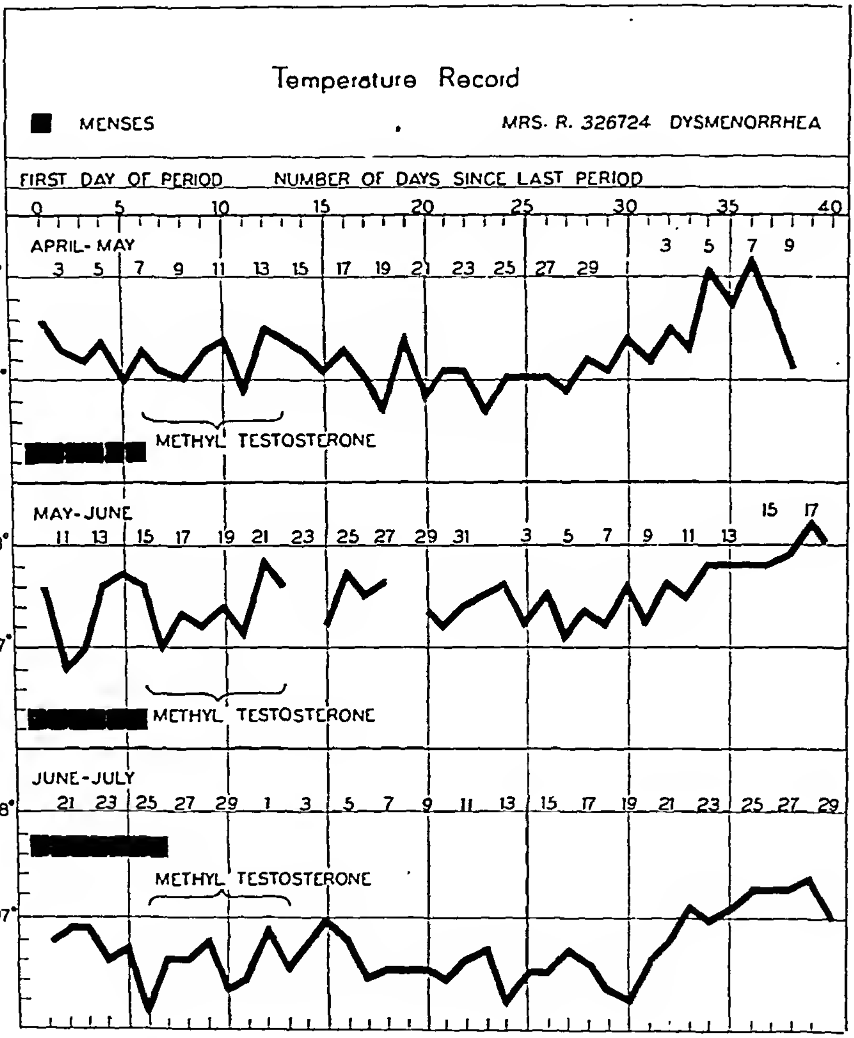

FIG. 7. The administration of methyltestosterone for the alleviation of dysmenorrhea does not produce an elevation of the temperature during the estrogenic phase. It often results in a prolongation of the cycle and suppression of ovulation.

three years. Our routine is to prescribe 30 milligrams of methyltestosterone orally each day beginning on day 7 of the cycle and continuing for eight days, until a total of 240 milligrams has been given during the cycle. The subsequent menstrual period is delayed considerably but it is likely to be

painless or associated with only moderate discomfort. Many of the cycles are anovulatory in character. The pattern of the basal temperature curve resembles that of the estrogenic phase. The general level may be somewhat lower than in the natural cycle. A rise in the temperature may occur at the end of a prolonged cycle. It can be concluded that testosterone does not produce a rise in body temperature (Fig. 7).

The third part of this presentation is a study of ovarian function following the surgical removal of the uterus. There are ample clinical data to show that ovaries may continue to function normally for many years following the careful surgical removal of the uterus in young women. However, some doubt has been cast on this supposition because, in many instances, symptoms of the climacteric develop within one or two years following hysterectomy. It is possible that this may be the result of the age of the individual and a lack of care in conserving the ovarian blood supply. Furthermore, there is some experimental evidence that the uterus is necessary for the normal metabolism of progesterone. Basal temperature studies provided a useful tool for obtaining new data concerning this problem.

In an attempt to study the ovarian cycle in the absence of the uterus, the following experiment was carried out. Twelve women who had complete hysterectomies for a variety of pathologic conditions, with the retention of one or both normal ovaries, started basal temperature graphs after they recovered following their surgical procedures. All had normal ovarian function and typical temperature curves prior to their operations. Urinary pregnandiol was determined for a 24-hour period once a week. The patients were followed for at least four months.

Figure 8 depicts a portion of the temperature records kept by four patients following the removal of the uterus. The general pattern of the curve is typical of normal ovarian activity recurring at approximately the same interval as the patient experienced when she was menstruating periodically prior to the removal of the uterus. The length of the temperature elevation was approximately fourteen or fifteen days, the average duration of corpus luteum activity.

Progesterone metabolism, as measured by the urinary output of pregnandiol during a 24-hour period was not altered. Minute quantities were present during the low level of the temperature prior to ovulation. These small amounts are found in normal women during the estrogenic phase of the cycle and may represent adrenal activity. Considerable amounts, ranging from 5 to 17 milligrams in a 24-hour period, were recovered during the phases of elevated temperature.

We concluded that follicles grow to maturity, rupture, and become functional corpora lutea which have a normal life cycle even in the absence of

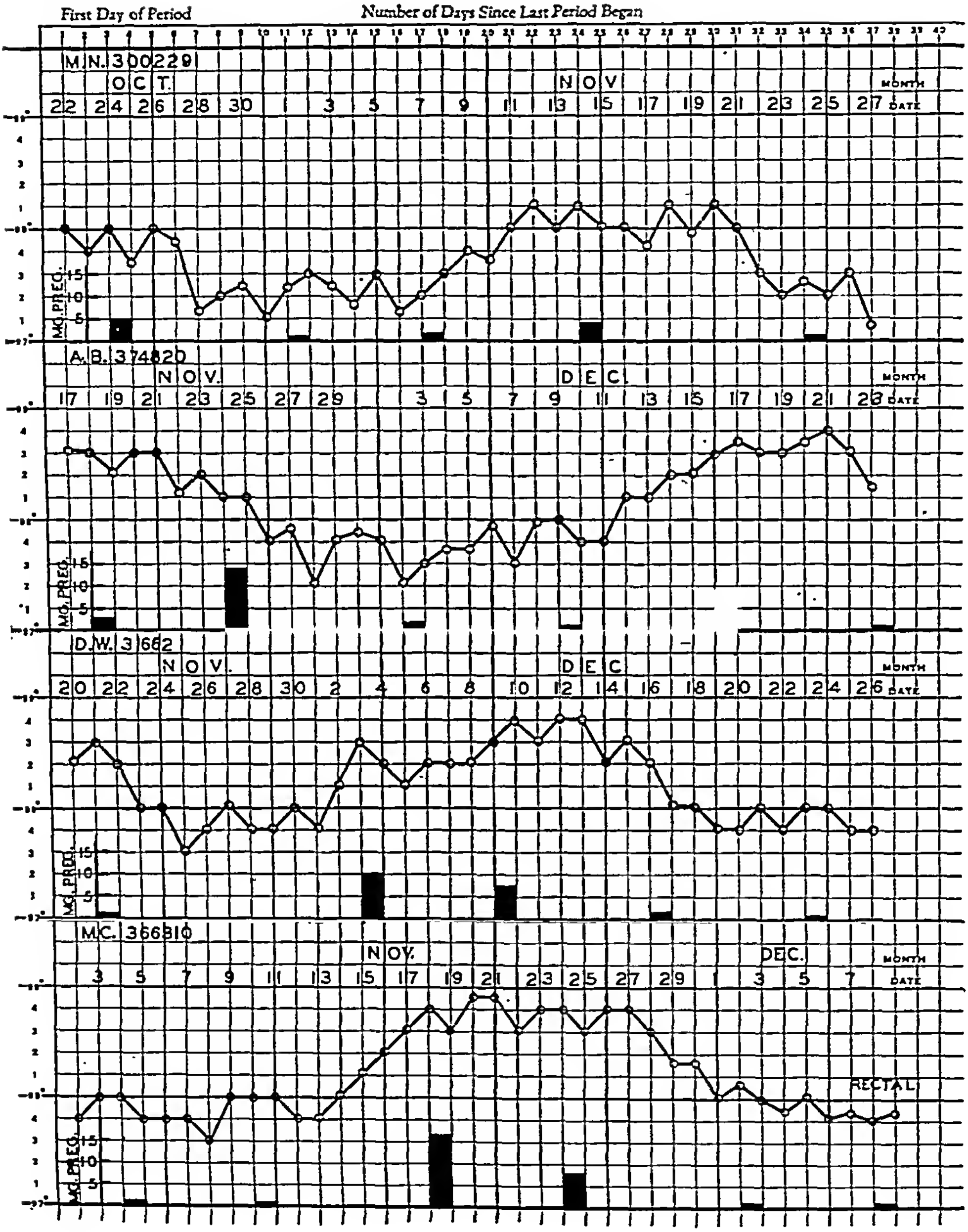

FIG. 8. Natural ovarian cycle in 4 young women each of whom had a complete hysterectomy. The temperature graph indicates a normal estrogenic phase and a normal luteal phase. Pregnandiol in the urine (black columns) was absent during the estrogenic phase and present in normal amounts during the luteal phase indicated by the elevated temperature.

the uterus. In the human the uterus is not necessary for a normal hormonal cycle. These patients are still being followed to determine if hysterectomy performed with care to safeguard the ovarian circulation interferes with the natural length of life of the ovaries.

DISCUSSION

The rise in basal body temperature during the luteal phase of the normal ovarian cycle is brought about by progesterone elaborated by the corpus luteum. The artificial reproduction of the normal hormonal cycle by the substitution of estrogens and progestins results in a temperature curve simulating that of the natural ovarian cycle. Progesterone can be identified positively as the factor responsible for the rise and maintenance of the elevated temperature in the luteal phase of the cycle.

An important consideration is the relationship of temperature rise and ovulation. The actual rupture of the follicle is unlikely to contribute to the temperature rise, for it appears to be insufficient trauma to disturb the temperature curve. In all likelihood the rise is caused by progesterone. If so, then the rise follows the rupture of the follicle and is dependent on the luteinization of the follicle wall, unless luteinization and progesterone production begin prior to the release of the ovum.

In the past we have regarded the luteinization of the follicle as beginning after follicle rupture and proceeding rather slowly. However, in the light of the fact that pregnancies do follow coitus limited to the phase involving the rise of temperature, and the excellent observations of Greulich correlating early corpora lutea and temperature changes in the human, we may have to reconsider this idea. It is possible that luteinization actually begins during the final phase of rapid growth of the follicle involving, according to Corner, the last eight or ten hours preceding ovulation. This final spurt of growth in the follicle about to rupture could be associated with luteinization.

Our concept concerning the theca interna cells has changed. Corner (8) from studies on the rhesus monkey and Greulich (6) from observations of early human corpora lutea concluded that the theca interna cells contribute significantly to the formation of the corpus luteum and that they continue to add to it throughout its functional life. These cells as well as the granulosa cells probably produce progestin. It is entirely possible that the luteinization of the follicle may begin in these theca interna cells prior to follicle rupture and continue at a rapidly increased rate following ovulation. If this were so it would be possible to account for ovulation taking place with the rise in temperature rather than at its lowest point prior to the rise. Such a concept would explain fertile matings during the rise in the temperature curve. It would not be necessary to theorize an increased life span for

the ovum. Obviously, many variations can occur in a physiologic process as carefully timed as the reproductive function.

CONCLUSIONS

Additional experimental evidence has been presented that progesterone is responsible for the rise in body basal temperature during the luteal phase of the normal ovarian cycle in women. Ovulation in many, if not in most instances, occurs with the rise in the temperature rather than at its lowest point prior to the rise. It is probable that follicle luteinization begins in the theca interna cells during the stage of rapid growth just prior to ovulation and becomes accelerated with the rupture of the follicle. This explanation would account for the onset of the rise in body basal temperature prior to ovulation.

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A FLUOROMETRIC METHOD FOR THE CLINICAL DETERMINATION OF ESTRONE AND ESTRADIOL*

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INTRODUCTION

THE biological assay for estrogens based on the vaginal response in ovariectomized rats was first standardized by Kahnt and Doisy (1). This method has been used by many investigators for the estimation of estrogenic activity in various biological fluids. However, biological assay has the disadvantage of being long, expensive and subject to variations inherent in animals. It also necessitates the maintenance of a large animal colony.

Several investigators have proposed colorimetric chemical methods for estimating the estrogen content of urine in order to avoid biological assay (Kober (2), David (3), Talbot et al. (4), Szego and Samuels (5)). The urine of normal males and females contains such small concentrations of estrogens that the methods proposed usually lack sufficient sensitivity. This necessitates the use of large quantities of urine, so that nonspecific urinary chromogens interfere with accurate determination.

During the past several years, fluorometric methods have been perfected to determine minute amounts of thiamine, riboflavin (6) and some of the antimalarial drugs (quinacrine, quinine and chloroquine) (7). In the isolation and purification of estrone, Marrian (8) pointed out that when concentrated sulfuric acid is added to estrone, an orange color with greenish fluorescence is produced. Bierry and Gouzon (9, 10) attempted to use this fluorescent phenomenon for the qualitative detection of estriol in pregnancy urine. Slight modification of this reaction forms a color which is the basis of some of the colorimetric methods found in the literature (Kober (2)). While the present work was in progress, Finklestein, Hestrin and Koch (11) presented a quantitative fluorometric method for the estimation of crystalline estrone, estradiol and estriol. This method depends upon the formation of a fluorescent substance when phosphoric acid is added to dry

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[1] Part of this investigation was done during the tenure of a "Post War Residency of the Rockefeller Foundation."

crystals of the estrogens. However, the sensitivity is still not great enough for its general application without the use of large quantities of urine. Furthermore, the method had not been adapted to biological fluids.

A preliminary note on a fluorometric method for determining estradiol and estrone using the principle of Marrian has been presented (Jailer (12)). The great sensitivity of the method makes it possible to detect as little as 0.05 microgram of crystalline estrone. Data were offered to show its applicability to urine. Bates and Cohen (13) presented a somewhat similar method on pure crystalline natural estrogens, but gave no application of their method to the assay of biological fluids.[2]

The purpose of this communication is threefold; to present the analytical method in detail; to show evidence of its reliability and to present data on estrogen excretion in various normal and pathological conditions.

METHODS AND MATERIALS

Estrone, estradiol and estriol standards are dissolved in methyl or ethyl[3] alcohol in the concentration of 10 milligrams per 100 milliliters. From these solutions, working standards containing 1.0 microgram per milliliter are made for estrone and estradiol and 4.0 micrograms per milliliter for estriol.

All organic solvents are redistilled before use, and all solutions are stored in glass stoppered bottles. The glassware must be scrupulously clean. Dow-Corning stopcock grease is employed. These precautions must be observed to prevent the appearance of nonspecific extraneous fluorescence.

For maximum fluorescence of the standards, 8.0 ml. of 60–70 per c nt (by volume) sulfuric acid (Mallinckrodt, A. R., low nitrogen) are added to 0.2–0.5 ml. of alcohol containing various amounts of estrogens. This is stirred well with a rod and placed in a boiling water bath for exactly 5 minutes, cooled and the fluorescence read in a Coleman photofluorometer, Model #12. The B-2 and PC-9A filter combination is used. There is a quantitative relationship between steroid concentration and galvanometer reading (Fig. 1). For estrone and estradiol the range is between 0.05 and 0.25 microgram; however, the fluorescence of estriol is about one-twentieth that of the former two.

Several other steroids show this same reaction with sulfuric acid. β-estradiol fluoresces to the same extent as α-estradiol. Testosterone, progesterone and desoxycorticosterone fluoresce to a somewhat lesser degree. Cholesterol fluoresces very slightly, 20 micrograms giving the same fluores-

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[2] Bates and Cohen (1948) have now utilized this principle in the estimation of estrogen in pregnant mare urine (personal communication).

[3] Originally methyl alcohol was employed because of the greater solubility of the estrogens in it. However, it has been discovered recently that several batches of methyl alcohol, even after redistillation, contain substances which inhibit this fluorescence. No such phenomenon has occurred with redistilled ethyl alcohol.

cence as 0.05 microgram of estrone. The reaction has been performed with androsterone, dehydroandrosterone and pregnandiol in concentrations as high as 200 micrograms with no photoluminescence. These steroids have also been added to aliquots of urine which were analyzed by the method described below. Even though testosterone and progesterone fluoresce,

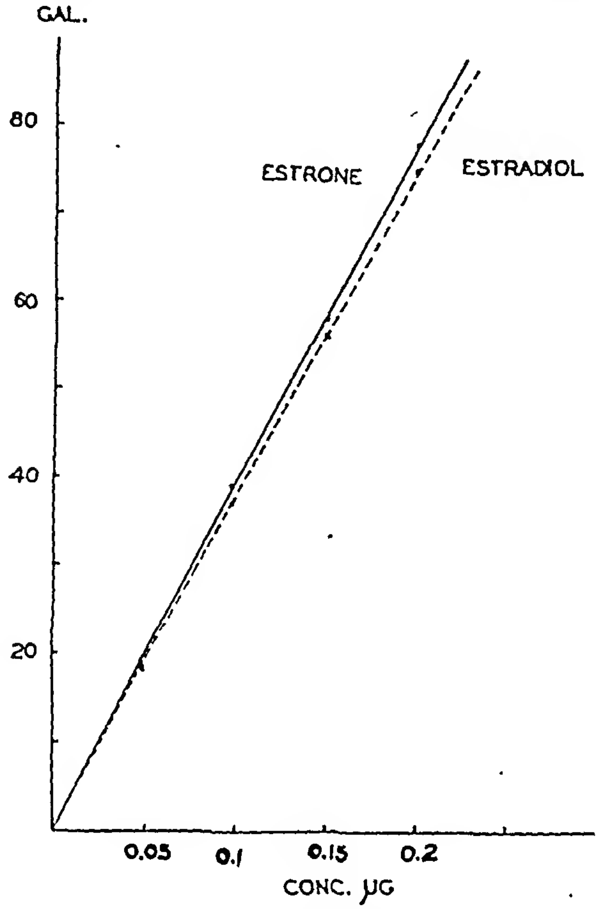

FIG. 1. Relationship between concentration of estrone and estradiol in micrograms and galvanometer readings (with B-2 and PC-9A filter combination).

when added to urine and extracted as described below, they do not interfere with the determination of estrogens. When 80 micrograms of desoxycorticosterone were added to urine there was some increase in the fluorescence, amounting to but a fraction of a microgram (Table 1).

Since the reaction with sulfuric acid is not completely specific, an extraction method was employed which separates the estrogens from other hormones which may also fluoresce. Use was made of the fact that the estrogens are soluble in alkali as contrasted to the other steroids mentioned, which are not. The method is a modification of the procedure of Bachman and Petit (14). It is as follows:

1. From 10 to 20 ml. of urine are hydrolyzed with 1 to 2 ml. of concentrated HCl, boiled for 7 to 10 minutes and cooled rapidly. (For pregnancy urine smaller aliquots are used.)

2. Transfer to a separatory funnel and saturate with NaCl.
3. Extract three times with benzene (A. R.; thiophene-free) using 25, 15 and 10 ml., respectively. (The interface gel should be saved as it contains some estrogen.)

TABLE 1. NONINTERFERENCE OF OTHER ADDED STEROIDS WITH THE FLUOROMETRIC METHOD

Urine (number)	Steroid Added	Amount (micro-grams)	Galvanometer		Concentration of Estrogen in Aliquot (micrograms)
			B-2	B-1	
1	None	None	36	28	0.13
	Pregnandiol	200	40	29	0.14
2	None	None	21	12	0.07
2	Progesterone	200	23	14	0.07
3	None	None	75	35	0.27
3	Androsterone	100	73	33	0.26
3	None	None	76	35	0.28
3	Cholesterol	200	75	35	0.27
4	None	None	47	17	0.25
4	Desoxycorticosterone acetate	80	65	22	0.36
5	None	None	10	4	0.03
5	Testosterone	80	10	3	0.03

4. The combined benzene fractions are washed with distilled water, twice with 9 per cent Na_2CO_3, and again with water using 15 ml. for each washing. (This extracts the estriol.)
5. The benzene fraction is extracted four times with 2 normal NaOH using 20, 15, 10 and 10 ml., respectively.
6. The combined NaOH fractions are combined and acidified with 15 ml. of concentrated HCl.
7. Extract three times with benzene, 20, 15 and 15 ml.
8. The combined benzene fractions are washed with water, Na_2CO_3, and again with water, using 15 ml. for each washing.
9. The benzene is freed of water, and evaporated to dryness in a beaker.
10. 1.0 ml. of alcohol is added to the beaker.
11. A 0.7 ml. aliquot is transferred to a test tube and 8.0 ml. of 60 to 70 per cent H_2SO_4 added and stirred well with a fluted stirring rod. The tube is placed in a boiling water bath for 5 minutes.
12. Cool, transfer to cuvettes and read in photofluorometer.

In all determinations, it has been found necessary to run a reagent blank (dummy) consisting of 10 ml. of distilled water which is extracted in the

same manner. This is set at zero in the fluorometer. Recoveries (0.2 microgram and 0.5 microgram of estrone or estradiol) from water and urine are also run. These have been found to be fairly constant ranging between 50

TABLE 2. RECOVERY OF KNOWN AMOUNTS OF ESTRONE AND ESTRADIOL WHEN ADDED TO EITHER WATER OR URINE AND EXTRACTED AS DESCRIBED

(Micrograms Added)	Substance	Performed Estrogen	Total Estrogen	Estrogen Recovered	Per Cent Recovery
Estrone					
0.2	Water	0	0.104	0.104	52
0.2	Water	0	0.10	0.10	50
0.5	Water	0	0.32	0.32	64
0.5	Water	0	0.28	0.28	56
0.2	Urine	0.089	0.179	0.09	45
0.2	Urine	0.110	0.220	0.11	55
0.5	Urine	0.38	0.66	0.28	56
Estradiol					
0.2	Water	0	0.10	0.10	50
0.2	Water	0	0.12	0.12	60
0.2	Urine	0.27	0.38	0.11	55
0.2	Urine	0.26	0.36	0.10	50

and 60 per cent (Table 2). Standards of 0.2 microgram are set up at Step #11[4] and read against the absolute blank which consists of alcohol and sulfuric acid.

It has been noted that the color of the fluorescence of crystalline estrone and estradiol is yellowish green, whereas that of urinary extracts may show a whitish tinge as well. Therefore, an attempt was made to attain greater selectivity with various filter combinations. The maximum fluorescence with crystalline estrogens occurs with an exciting light of 435 millimicrons[5] (filter B-2). Changing the exciting wave length to 365 millimicrons (filter B-1) will decrease the galvanometer reading to practically zero (Table 3). However, the nonspecific fluorescence of the reagent blank is practically identical at the different wave lengths. It was also noted that the B-1 reading of urines containing very small amounts of estrogen is a much higher percentage of the B-2 reading than normal urines. Urines containing

[4] If necessary the sensitivity can be increased slightly by using sulfuric acid of higher concentration, e.g. 80 to 90 per cent. However, the concentration used should be well standardized.

[5] Since the B-1 filter contains a grid which allows 30 per cent transmission, a similar grid, of $\frac{1}{8}$ inch wire mesh, has been interposed in the B-2 filter. All values mentioned have been with this grid.

high amounts of estrogen, e.g. pregnancy urine or stallion urine, showed practically no fluorescence at 365 millimicrons. Thus, by reading the fluorescence of the urinary extract at the two wave lengths, one can determine the amount of estrogen and nonspecific fluorescence. The formula adopted for the correction factor is similar to the one proposed by Stimmel (15): $\frac{\text{Unknown } B_2 - B_1}{\text{Standard } B_2K(1-(B_1B_2)}$. B_2 is the galvanometer reading with the B-2 filter; and B_1, the reading with the B-1 filter; K is the constant of the ratio of the value of the reagent blank with the two filter systems. For these purposes it is unity.

For the biological assay of the estrogenic content of urine, the same extraction procedure as described above was employed, except for the stallion urines, where ether was used as the organic solvent. Girard separation was performed to separate the extract into ketonic (estrone) and nonketonic fractions (estradiol) in seven of the nine assays, and each assayed separately according to the method of Kahnt and Doisy (1). An aliquot was also saved and assayed for total estrogenic activity.

Twenty-four hour specimens were collected for the majority of cases; however, in some, accurately timed samples were analyzed. In most cases, completeness of collection was checked by creatinine determinations. Since recoveries of added amounts of estrone and estradiol averaged 50 per cent, it was assumed that recovery of the endogenous estrogens was similar; consequently all values reported in this communication have been computed on that basis and are twice the actual values obtained. This was not done when the chemical method was compared with biological assay; here it was assumed that the percentage recovery was identical in both.

Duplicate analyses of different aliquots of the same urine specimens have been made, on the same day and on different days The error was within 20 per cent.

The length of time necessary for the completion of a biological assay for estrogens depends upon the number of test animals available, but at best it takes several days. With the fluorometric method, using small aliquots of urine it is possible to analyze as many as five urines, together with a reagent blank and a recovery experiment, in one day.

RESULTS

After performing recovery experiments with estrone and estradiol from water and urine, data were accumulated to ascertain the reliability of this method.

1. *The Urinary Estrogen Excretion in Normal Women.* The urinary excretion of estrogens has been shown to be high in certain clinical conditions

TABLE 3

Substance	Galvanometer Reading B-2 435 millimicrons	Galvanometer Reading B-1 365 millimicrons	Per Cent Nonspecific Fluorescence
Estrone 0.2 micrograms	80	4	5
Estradiol 0.2 micrograms	76	5	7
Estriol 2.0 micrograms	40	2	5
Extract of urine from normal female	a) 56	35	63
	b) 95	50	56
	c) 87	44	51
Extract of urine from normal male	a) 70	29	42
	b) 78	37	48
	c) 40	20	50
Extract of urine from woman in 9th month of pregnancy	a) 25	0	—
	b) 54	9	17
	c) 46	0	—
Extract of stallion urine	a) 62	2	3.2
	b) 82	7	8.5
	c) 100	12	12
Extract of urine from case of craniopharyngioma	40	30	75
Extract of urine from woman past the menopause	16	10	75
Extract of urine from woman past the menopause	10	4	40
Extract of urine from woman past the menopause: (estrone added)	22	4	18
Reagent blank	a) 14	11	80
	b) 15	13	87
	c) 14	12	86
	d) 14	12	86
Nonpurified benzene	a) 32	32	100
	b) 87	94	108
Nonpurified ether	a) 25	21	84
	b) 14	13	93

and low in others, consequently an attempt was made to estimate the urinary excretion of estrogens by the fluorometric method and compare the results obtained with those reported in the literature.

The urine of two normal young women has been studied through three menstrual cycles by daily analyses for estrone and estradiol. The results are shown in Figure 2. It can be seen that there is a peak excretion at mid-interval and another just preceding menses. This is in agreement with results published by Gustavson *et al.* (16), Werner (17), Gallagher *et al.* (18) and others, as shown by bio-assay. These values were correlated with basal body temperature and it is shown that the mid-interval estrogen peak immediately precedes the rise in temperature. In one cycle, there was no abrupt rise in body temperature at mid-interval nor was there a sharp fall with menses; in this case there was no sharp peak of estrogen excretion as had been experienced during the preceding menstrual cycle (Fig. 2). In another menstrual cycle studied by daily urinary estrogen excretion, a "picket fence"-like graph was obtained, thus differing somewhat from the usual smooth graphs obtained by other investigators.

2. *Urinary Estrogen Excretion After the Administration of Estrogen.* It has been demonstrated by several investigators that the parenteral or oral administration of estrogens results in an increased excretion of these steroids in the urine. As shown in Table 4, two milligrams of estradiol benzoate were administered intramuscularly on two successive days to one normal young male (Case 1) and five milligrams of estrone sulfate were given orally on two successive days to another (Case 2). In addition five milligrams of estradiol in oil were given in single injections to each of two women in the menopause (Cases 3 and 4). Twenty-four hour specimens of urine were analyzed prior, during and after the administration of the estrogens. There was an increase in the urinary excretion of estrogens comparable with the results obtained by previous workers (Pincus and Pearlman (19); Heard and Hoffman (20)). The nonspecificity of the reaction suggested that the fluorometric method might determine degradation products of estrone and estradiol that are biologically inactive, consequently one of these urine specimens was analyzed by bio-assay as well. The results are compared in Table 5 (d) and it may be seen that they are in remarkably good agreement.

3. *Comparison of the Fluorometric Method with Biological Assay.* Urines from six patients were extracted according to the described method. Aliquots were taken for chemical analysis and the remainder assayed according to the method of Kahnt and Doisy. The correlation appears to be reasonably good considering the degree of error inherent in the biological method, the incompleteness of the Girard separation and the loss of biological activity in that procedure (Table 5).

The correlation between the bio-assay and the chemical method with the three samples of stallion urine was not as good as was the correlation in

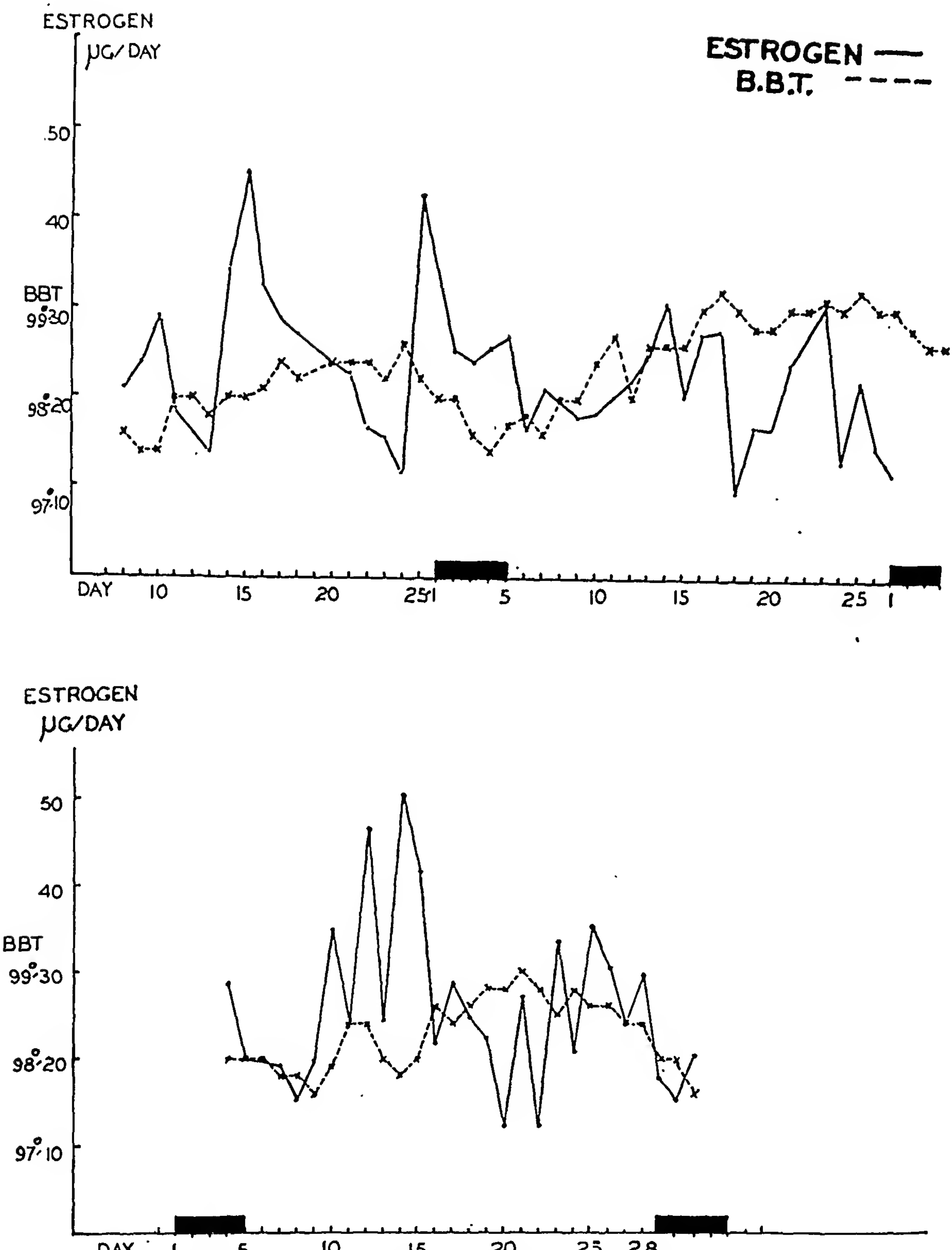

FIG. 2. Daily urinary excretion of estrogen (estrone and estradiol) in two normal young women. Blackened area represents menstruation. The unbroken line is estrogen excretion and the broken line is basal body temperature.

TABLE 4. THE EFFECT OF ADMINISTERED ESTROGEN ON THE EXCRETION OF FLUORESCENT SUBSTANCES IN THE URINE

	Case 1 (normal male)		Case 2 (normal male)		Case 3	Case 4 (Two women in the menopause)	
Day	Estrogen administered intramuscularly	Fluorescent substances in urine (micrograms)	Estrogen administered intramuscularly	Fluorescent substances in urine (micrograms)	Estrogen administered intramuscularly	Fluorescent substances in urine (micrograms)	
1		21.6		26.2		27.5	26.9
2	Estradiol benzoate (2 mg.)	19.2	Estrone sulfate (5 mg.)	88.0	Estradiol (5 mg.)	196.3	129.5
3	Estradiol benzoate (2 mg.)	46.7	Estrone sulfate (5 mg.)	116.0		62.2	47.1
4		52.0		61.7		41.4	29.0
5		70.3		23.2			

urine from human beings. There are several reasons for this discrepancy. Benzene was used as the organic solvent for the chemical method and ether was employed for extracting the estrogens for bio-assay.[6] The high concentration of estrogens in the extract necessitated great dilution of the final alcoholic extract in order to obtain a reading on the galvanometer scale. Thus, perhaps accuracy was impaired by the extreme dilution. These results are presented in Table 5.

4. *Estrogen Excretion in Various Normal and Pathological States.* The remainder of the data to be presented in this communication are concerned with the urinary excretion of total estrone and estradiol in various normal and pathological conditions. These are summarized in Table 6.

Normal males excrete between 20 and 26 micrograms of estrone within a 24-hour period. These values, obtained by the fluorometric method, are somewhat higher than those found by biological assay although values of this magnitude have been obtained occasionally (Gallagher et al. (18)). Children of both sexes excrete but small amounts. Women in the menopause excrete about the same amounts as women with amenorrhea, or

[6] I wish to thank Dr. Louis Levin for assaying the stallion urines and placing the results at my disposal.

TABLE 5. COMPARISON OF FLUOROMETRIC METHOD WITH BIOLOGICAL ASSAY, AS PERFORMED BY THE METHOD OF KAHNT AND DOISY

Urinary Excretion of Estrogens

Urine from	Fluorometric Method	Biological Assay					
		Total		Ketonic Fraction		Nonketonic Fraction	
	Micrograms	Micrograms	R.U.	Micrograms	R.U.	Micrograms	R.U.
Patient							
A.	23.1		20				
B.	12.4		20				
C.	220.0	128	490	94	94	34	280
D.	104.0	111	278	94	94	17	141
E.	136.0	114	400	84	84	30	250
F.	182.0	95	328	82	82	13	104
Stallion	*mg./liter*	*mg./liter*	*R.U./liter*	*mg./liter*	*R.U./liter*	*mg./liter*	*R.U./liter*
A.	10.00	29.1	52,000	26.0	26,000	3.1	26,000
B.	12.24	16.0	40,000	13.6	13,600	2.4	20,000
C.	7.74	11.08	20,800	10.0	10,000	1.08	9,000

normal women between peaks of excretion. Some of the older women, past the menopause, excrete much smaller amounts. One patient, aged 65, many years past the menopause, excreted no detectable estrogen. However, several women in this age group excreted as much as 20 to 30 micrograms per day.

Gravid women during the last trimester of pregnancy excrete large amounts of estrogen, as has been reported by many investigators (Zondek (21a), Smith and Smith (22)). These values obtained by chemical methods are lower than those obtained by bio-assay for comparable periods of gestation. It should be pointed out that estriol is not included in these results.

The highest values attained were in stallion urine, as described by Zondek (21b) and by Levin (23).

In only one case of gynecomastia was the estrogen excretion slightly elevated. This occurred in a 38-year-old male with typical "Klinefelter-Reifenstein-Albright syndrome" (24).

The urinary excretion was low in pituitary and adrenal hypofunction. It appeared normal in a young woman with Cushing's syndrome; but was exceedingly high in two children with virilizing adrenal tumors. In one, the urinary excretion of estrogens, after removal of the tumor, fell from the preoperative level of 114 micrograms to 6.6 and 4.4 micrograms per day.

DISCUSSION

A fluorometric method for the clinical estimation of estrogens (estrone plus estradiol) in urine has been presented. It eliminates the use of the biological assay. The great sensitivity of this fluorescent phenomenon makes it possible to assay urine containing physiological amounts of hormone using but small and convenient samples of urine. In standard solutions the range of estrogen concentration which can be determined in the Coleman fluorometer is between 0.05 and 0.25 microgram of estrone and estradiol. However, being a fluorometric method, it is open to various disadvantages of this general type of method. Cork and rubber stoppers must be avoided in order to prevent the appearance of extraneous fluorescence. New batches of reagents (especially methyl alcohol and sulfuric acid) must be checked before use, as it has been found that occasionally they may contain substances which inhibit fluorescence.

The lack of specificity is also a disadvantage. Other substances fluoresce but to a lesser degree. Finklestein *et al.* (11) and Bates and Cohen (13) have found that cholesterol does not fluoresce. It may very well be that the minute amount of fluorescence obtained with cholesterol in this study was due to a contaminant. It appears that the property of fluorescence depends upon the presence of an unsaturated bond in ring A. The substances which fluoresce, when treated with sulfuric acid, all seem to have this characteristic in common. Utilizing the differential solubility of the acidic estrogens in alkali, an attempt is made to exclude other steroids which may be found in urine. Therefore, we are actually determining lipoid-soluble phenols which, on the addition of sulfuric acid, fluoresce at 435 millimicrons and not at 365 millimicrons.

The phosphoric acid method of Finklestein *et al.* (11) does not have the great sensitivity of the sulfuric acid procedure. These workers have presented no data on the assay of urinary extracts. Bates and Cohen (13) have reported a similar method using 90 per cent sulfuric acid. The high concentration of the acid when added to urinary extracts may cause charring which colors the solution. The lack of sensitivity in their method appears to be due to their use of a filter system which does not give maximum transmission of the exciting light. Bates and Cohen (personal communication) have devised a very simple method for determining the amount of estrogen in the urine of pregnant mares, where the concentration of estrogens is high. The procedure using 90 per cent sulfuric acid does increase the sensitivity of estriol, so that with the proper filter system (B-2; PC 9A) it is ten times more sensitive than with 60 per cent sulfuric acid (unpublished data). Thus, with 90 per cent sulfuric acid, estriol has one-half the sensitivity of estrone or estradiol.

The data in this communication are presented with the view of showing the reliability of the fluorometric method. It has been demonstrated that

TABLE 6. URINARY ESTROGEN EXCRETION IN VARIOUS NORMAL AND PATHOLOGICAL STATES

Status	Number	Age (Years)	Remarks	Estrogen Excretion Micrograms/24 hours
Males	1	32	Normal	21.6
	2	31	Normal	26.2
	3	37	Normal	26.0
Children	1	10	Urethral stricture	6.5
	2	5	Cryptorchism	3.0
	3	5	Enuresis	4.2
	4	7	Hydrocele	5.3
Women during and past the menopause	1	54	Irregular and scanty menses	10.6; 26.6; 23.4
	2	50	Irregular and scanty menses	16.7; 13.7
	3	60	4 years past the menopause	5.9
	4	65	7 years past the menopause	0
Gynecomastia	1	27	No liver pathology	24.0
	2	54	Carcinoma of breast	17.3
	3	38	"Klinefelter" syndrome; 17-ketosteroids, 6.9; gonadotropins, 50 m.u.; small testes	32.8
Hypopituitarism	1	29	Prepuberal hypopituitarism	9.3
	2	41	Chromophobe adenoma	14.7
	3	41	Early Simmonds' disease	9.1
	4	40	Simmonds' disease	0; 0; 2.0
Adrenal disease	1	42	Classical Addison's disease, maintained on DCA	9.6
	2	27	Cushing's syndrome; adrenal hyperplasia 17-ketosteroids, 23 and 17 mg.	19.0
	3	7½	Virilizing tumor; 17-ketosteroids, 175 mg.	296.0
	4	6	Virilizing tumor; 17-ketosteroids, 189 mg.	114.0
			After removal of tumor	6.6; 4.4
"Primary ovarian agenesis"	1	18	Failure to grow; amenorrhea; minimal breast development; infantile uterus and genitalia	5.8

TABLE 6 (*continued*)

Status	Number	Age (Years)	Remarks	Estrogen Excretion Micrograms/24 hours
	2	17½	Height 4′10″; no breast development; amenorrhea; no pubic or axillary hair; infantile uterus: receiving stilbestrol	8.9
Ovarian tumor	1	64	Mass in adnexa; vagina and endometrium showed estrogenic stimulation	20.6
			Given x-ray treatment	4.8; 6.3
Pregnant women	1	20	8th month	365.0
			After zinc dust hydrolysis of specimen	549.2
	2	25	9th month	592.8
	3	30	10th month, 2 days before delivery	1500.0
Stallions	1		Normal	12240 (micrograms per liter)
	2		Normal	7740
	3		Normal	10000

the values obtained with this method are in the same order of magnitude as those reported in the literature in similar clinical conditions by biological assay. The administration of exogenous estrogens results in an increase in fluorescent substances in urine. Finally, satisfactory correlation between biological assay and the fluorometric method has been obtained in urines from six human subjects. The concentration of estrogens was so high in the urines from three stallions that serial dilutions of the urine for chemical analyses was necessary. This may have impaired the accuracy of the fluorometric analysis. Further comparison showed much better correlation between the two (unpublished data). This correlation is somewhat similar to that obtained between the capon test for androgen and the colorimetric method for 17-ketosteroids (Holtorff and Koch (25)).

Great difficulty has been encountered in applying this method to the determination of estriol in urine. It appears that the urinary chromogens cannot be separated from the estriol fraction by differential solubility. Difficulties thus far unsolved have also been met in attempting to adapt this method to blood and tissue. Szego and Roberts (26) have shown that

the estrogens are bound to proteins and that the usual protein precipitates do not free the estrogens from the protein binding. Thus, hydrolysis and extraction with organic solvents must be performed in the presence of proteins. However, work is in progress to apply this method to blood and tissue.

It appears that the values for the urinary excretion of estrogens obtained by the fluorometric method are somewhat higher than those reported in the literature with biological assay. It is especially so in urine from normal males and in urine from women in the menopause. This may be because some unknown nonestrogenic substances, in the acidic fraction, may fluoresce similarly to estrone and estradiol. Another possibility is that the filter system used may not be optimum, so that some of the nonspecific fluorescence is being recorded. The latter possibility is being investigated with an interference selective filter (524 millimicrons) which is close to the wave length of maximum transmission of the fluorescent light (Bates, personal communication).

CONCLUSIONS

1. Sulfuric acid in 60 to 70 per cent concentration, when added to alcoholic solutions of estrone and estradiol in the presence of heat, results in the development of a fluorescent compound.
2. A fluorometric method for the determination of estrone and estradiol in urine is presented, making use of this phenomenon.
3. Data in various normal and pathological conditions are made available to show the possible clinical application of the method.

ACKNOWLEDGMENT

The author wishes to express his appreciation to Dr. Earl T. Engle for his encouragement throughout the course of this work and to Mrs. Ruth S. Finkel for her technical assistance.

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Edwin J. Kepler
1894–1947

Obituary

★

EDWIN J. KEPLER

1894–1947

Dr. E. J. Kepler was born in Erie, Pennsylvania, on January 22, 1894. He received the B.S. degree from Pennsylvania State College in 1916. He was an army bacteriologist in the first World War and, after the war, attended the University of Minnesota, where he received the M.D. degree in 1924 and where, also, he was admitted to the honorary medical society, Alpha Omega Alpha. For eighteen months he was an intern in Philadelaphia General Hospital. Thereafter, until his death, he lived and worked in Rochester, Minnesota, first as a Fellow of the Mayo Foundation, then as a Consultant in Medicine at the Mayo Clinic and as Professor of Medicine on the faculty of the Mayo Foundation, Graduate School, University of Minnesota. He was married to Dr. Helen A. Mackeen in 1921 and he is survived by her and their two daughters, Mary and Marcia.

These vital statistics are important, but they give no picture of Kep, as he was called by his many friends. Kep had so many facets to his nature that he is difficult to describe. He was known on the Mississippi as an enthusiastic riverman—one who liked to pilot his boat through seldom used channels, who enjoyed catching bass on flies and who was quite happy with the company of his family and friends. He was known at home as a family man who loved being at home. His friends think that Mrs. Kepler must have despaired at times at keeping order with all of the photographic and sporting equipment with which Kep surrounded himself. He was known in the Mayo Clinic as the most brilliant man in his section. He was a conscientious clinician and would spend any amount of time and effort in attempts, usually successful, to ferret out the diagnosis in difficult cases. Although his main interest was in metabolic diseases, his knowledge of general medicine was extremely wide. His contributions to clinical knowledge of diseases of the suprarenal glands were brilliant. It was in this field that he was best known by his friends outside Rochester.

When what he could write would serve a purpose, he wrote. He knew what he meant to say and his knowledge of English composition was such that he knew whether he had succeeded or failed in saying it. He did not release his material until it satisfied his knowledgeable standard.

He was a member of the American Association for the Study of Goiter (now the American Goiter Association), the Association for the Study of Internal Secretions, the Central Society for Clinical Research, the American Diabetes Association, the Laurentian Hormone Conference, the Central Interurban Clinical Club, and other organizations.

His death on October 19, 1947, from a coronary occlusion came as no surprise since his family, his friends and he himself were well aware of his serious heart condition. He is missed by his family, his colleagues and his many friends who, nevertheless, can rejoice in the fine influence which he created and which will always live.

Edward H. Rynearson, M.D.

THE ASSOCIATION

FOR THE STUDY OF

INTERNAL SECRETIONS

ABSTRACTS OF PAPERS PRESENTED AT THE THIRTIETH ANNUAL MEETING

FRIDAY AND SATURDAY, JUNE 18 AND 19, 1948

Palmer House, Chicago, Illinois

ABSTRACTS ARRANGED ACCORDING TO NUMBER ON PRINTED PROGRAM

FRIDAY, JUNE 18

9:30 A.M. Red Lacquer Room

1. PSEUDOGLANDULAR DISTURBANCES.
Hugo R. Rony. Chicago, Illinois.

In general, hormonal effects depend on end-organ sensitiveness as well as on the amount of the active hormone. Normal variations in skeletal growth, mammary development, amount of body hair, basal metabolic rate, etc., are probably due to variations in end-organ sensitiveness rather than to variations in the amount of hormones produced by the glands. Abnormal levels of end-organ sensitiveness may produce clinical pictures that closely imitate glandular disturbances—unresponsiveness resulting in pseudoglandular deficiency, hypersensitiveness resulting in pseudoglandular hyperactivity. Pseudoparathyroid deficiency, in this sense, has recently been described by Fuller Albright and his associates.

In the present paper attention is called to other types of abnormal end-organ response and to ways and means to differentiate them from true glandular disturbances. Cases of abnormal end-organ response are described that imitate thyroid, male and female gonadal, adrenal, insular and hypophyseal deficiency and hyperactivity. The likelihood of such origin is suggested for certain other types of "glandular" anomalies.

Recognition of the nature of these conditions is of considerable practical importance in that it may enable us to avoid errors in interpretation and disappointments in glandular therapy.

2. SYNDROME OF CRYPTORCHIDISM, HEART DISEASE AND NEVOID DERMATOSIS. REPORT OF TWO CASES.
S. J. Glass. Department of Endocrinology, Cedars of Lebanon Hospital, Los Angeles, Calif.

Two young adult, mentally alert males presented essentially the same multiple congenital anomalies, characterized by:
(1) unilateral cryptorchidism
(2) heart disease, well compensated in both subjects
(3) webbing of the neck and flaring of the ears
(4) inferior musculature
(5) hyperkeratotic-like dermatosis.

After the cure of the cryptorchidism by organotherapy in one subject and by surgery in the other, the sexual and skeletal growth continued uneventfully to relatively complete maturity.

3. Constitutional Precocious Puberty Controlled by Androgen Therapy.
S. Charles Freed and Minnie Goldberg. San Francisco, Calif.

A girl of four and one-half years had typical changes of precocious puberty. Investigations including laboratory determinations and x-rays seemed to indicate that the disorder was of the constitutional type. Methyltestosterone by mouth was administered for over two years and was successful in preventing menstruation and in suppressing the breast development. Bone age studies showed that while growth was markedly accelerated, the epiphyses of the long bones were nevertheless sufficiently well open to dispel fears of closure before a desirable height is attained. We have concluded that androgen therapy was beneficial in checking the psychologically traumatic features of precocious puberty as well as combating the serious threat of dwarfed stature usually associated with this condition.

4. A Study of the Biological Activity and the Magnitude of Endogenous Androgen Production in a Case of Adrenogenital Syndrome.
Anne C. Carter and Ephriam Shorr. From The Russell Sage Institute of Pathology and the Department of Medicine, Cornell University Medical College and The New York Hospital, New York City.

Although it is generally appreciated that, in virilism resulting from adrenal tumors or hyperplasia, urinary 17-ketosteroid values provide little information as to the amount or biological activity of their adrenal precursors, it has been difficult to set up experimental conditions in man by which the extent of endogenous hormone production could be quantitated. A case of virilism due to bilateral adrenal cortical hyperplasia in a 17-year-old girl provided conditions favorable for such an evaluation.

To this end a variety of histological and metabolic indices were selected which are influenced in opposite fashion by androgens and estrogens. These included vaginal smears, endometrial and skin biopsies, changes in the clitoris, breasts and rate of hair growth, urinary citric acid, calcium and creatine, and urinary hormone assays. Estrogens were then given in ascending doses until the effects of the endogenous androgens were neutralized, as judged by these criteria. Complete neutralization required 400,000 RU (40 mg. ethinyl estradiol, orally) of estrogen daily. On the basis of previous experiments in this laboratory on the neutralizing ratios of estrogens and androgens, it was estimated that this amount of estrogen would neutralize 3300 mg. of testosterone propionate given intramuscularly.

5. Pseudohermaphrodism: Early and Late Recognition.
M. James Whitelaw. Southern Medical College, Dallas, Texas, and St. Monica Hospital, Phoenix, Arizona.

Two children under five years of age are described showing the typical physical and chemical findings of male and female pseudohermaphrodites respectively, which included an elevated 17-ketosteroid excretion. At operation a hyperplastic adrenal was removed from each patient, following which their physical characteristics approached normal. Studies done on the total white count as well as the leucocyte-lymphocyte ratio previous to, and following operation showed no deviation from the normal. The third case is that of a 12-year-old dwarf female pseudohermaphrodite, who had been brought up as a boy. There was remarkable masculine physiognomy and physique. A 24-hour course of epinephrine was given to determine its effect on 17-ketosteroid output. There was some evidence of an increase. A plea is made for early recognition and surgical treatment of these cases.

6. CLINICAL, LABORATORY, OPERATIVE, AND POSTMORTEM OBSERVATIONS IN INFANTS AND CHILDREN WITH MULTIPLE CONGENITAL MALFORMATIONS (TURNER'S SYNDROME, OVARIAN AGENESIS AND RELATED COMBINATIONS).

Frank L. Plachte. From the Endocrine Clinic, Children's Hospital, Los Angeles, Calif.

A group of 15 infants and children with webbing of the neck and other congenital malformations has been under investigation. Their age ranges from the newborn period to late childhood. The group includes cases of Turner's syndrome, ovarian agenesis or related complexes of multiple congenital malformations and developmental retardation. Two of the patients are boys. Two of the group have also been studied at autopsy.

Several of the cases show unusual clinical and laboratory manifestations not previously reported in the literature. The urinary tract has been studied by pyelography, at operation, or at autopsy in thirteen of the group, and eight patients show urinary malformations of various types.

7. A SYNDROME CHARACTERIZED BY HYPERCALCEMIA, CALCINOSIS, AND RENAL INSUFFICIENCY FOLLOWING PROLONGED INTAKE OF CALCIUM AND ALKALI.

Charles H. Burnett, Robert R. Commons (by invitation), Fuller Albright, and John E. Howard. Evans Memorial Hospital, Department of Medicine, Boston University School of Medicine, Massachusetts General Hospital, Department of Medicine, Harvard Medical School, Boston, and Johns Hopkins Hospital and University, Baltimore.

Data from six adult males have been collected. Each gave a history of prolonged periods (two to twenty-six years) of treatment for peptic ulcer or dyspepsia with large quantities of milk and absorbable alkalis. Pruritis or a complication of peptic ulcer were the usual reasons for hospitalization. At this time calcinosis, most frequently in the cornea or conjunctivae, was observed. Azotemia was a constant finding. Hypercalcemia without hypophosphatemia, and unaccompanied by hypercalcuria was demonstrated in five patients. Other less striking biochemical changes in the blood included moderately high serum carbon dioxide content and moderate hyperproteinemia without hyperglobulinemia. Evidence of severe renal disease was uniformly present; clearance measurements in three, demonstrated advanced insufficiency of all portions of the nephron. Two patients died and on one an autopsy was performed. Normal parathyroids and chronic pyelonephritis were demonstrated. In one of the four living patients parathyroid hyperplasia was demonstrated at operation. In another a normal kidney biopsy was obtained two years before renal failure occurred. Low calcium, low alkali regimens have produced symptomatic improvement and decreased serum calcium, nonprotein nitrogen, and carbon dioxide content.

8. HYPOPARATHYROIDISM, WITH MENTAL TROUBLES AND ECTODERMAL DISORDERS.

Manuel Villaverde. Habana, Cuba.

Idiopathic latent tetany is a rare condition. Nevertheless, the syndrome outlined in this paper is frequent (0.6% to 1% of the total consultations in the office). It is of hypoparathyroid nature, as proved by the clinical picture of latent tetany, the nervous irritability, the results of blood chemistry, the family endocrine background, and the impressive improvement of the patients during adequate therapy. These elements sustain the diagnosis of hypoparathyroidism in patients suffering from mental troubles frequently accompanied by ectodermal disorders, namely, decalcified and abraded teeth, and early appearance of gray hair. The nervous symptoms range from a troublesome nervousness, which we may call cycloid or schizoid, to anxiety, compulsive neurosis,

mania, crises of weeping, circular insanity, and sometimes schizophrenia. Patients may give a history of spasmophilia, cramps, or true tetanic crises. The gray hair, which is not different from common gray hair, appears before the thirties. The teeth become sensitive, and show decalcification and abrasion of the free borders; minute fractures sharpen the teeth making them look like a saw, and their borders appears translucent and bluish. Sometimes crises of greater sensitivity may announce an increase of the destructive process, which can shorten the teeth to half or less of their former size.

These three complaints—mental troubles, abraded teeth, and gray hair before the thirties—are the elements that constitute the syndrome, the study of which is to be encouraged because of its frequency, and the excellent results of therapy. Hyperestrogenism is often the cause of the syndrome. There are some cases with predominant mental symptoms.

9. Treatment of Far-Advanced Inoperable Carcinoma of the Breast with Estrogens and Androgens.

Samuel G. Taylor, III, Danely Slaughter, Frederick W. Preston. Chicago, Illinois.

Clinical results in the treatment of advanced inoperable carcinoma of the breast with various estrogens and androgens will be reported. The series includes 44 patients who were treated with estrogens and 30 patients treated with androgens over a two-year period.

The most favorable results with estrogen therapy were obtained in the older age group. Reduction in the primary tumor, in regional lymph gland metastases and in recurrences in the skin, with healing of ulceration occurred in some patients. However, there was occasional acceleration in tumor growth. In no patient given estrogen therapy were we able to demonstrate regression of osseous metastases, but in several patients there was no progression in these metastases during observation.

Androgen therapy produced regression in soft tissue metastases in several patients: however, improvement in osseous lesions was more frequent. Severe masculinization commonly occurred and six patients developed congestive heart failure requiring omission of androgen therapy.

In one patient a large carcinomatous ulceration disappeared during estrogen therapy but recurred in nine months. This did not respond to estrogen therapy a second time but regressed during androgen therapy.

A few patients were given desoxycorticosterone. No regression in the tumor was noted during this therapy.

We have not been able to predict whether estrogen will prove to be more efficacious than androgen therapy in any individual case. Estrogens are not ordinarily of value in the younger age group.

10. Hormonal Factors Involved in the Regulation of Basal Body Temperature During the Menstrual Cycle and Pregnancy.

Charles L. Buxton and William B. Atkinson. Departments of Obstetrics and Gynecology and of Anatomy, College of Physicians and Surgeons, Columbia University, N. Y., and the Sloane Hospital for Women, N. Y.

In order to ascertain the cause of the well known postovulatory rise in basal body temperature in normal cyclic women, six patients with amenorrhea and with little or no endometrial activity were given sequential therapy of estrogen followed by progesterone. The patients were followed by means of temperature charts and endometrial biopsy. The basal body temperature invariably rose significantly (three-fifths to one degree F.) during progesterone therapy.

The postovulatory temperature rise was also maintained and menstruation postponed ten to thirty days in normal women by the administration of chorionic gonadotropin. However, chorionic gonadotropin administered to a castrate following estrogen priming did not result in any significant change in basal temperature. It was assumed, therefore, that the maintenance of the postovulatory temperature rise by chorionic gonadotropin was due to its luteotropic effect and that here also progesterone produced the temperature response.

In order to determine whether the rise were maintained throughout gestation, three women (one through two pregnancies) kept temperature charts until delivery. One other is now in the sixth month. The temperature fell to its preovulatory level uniformly between the fourth and fifth month in all cases. This is in spite of the commonly supposed steady rise in progesterone activity as determined by pregnandiol excretion. Some conjecture, but no final explanation is offered for this interesting phenomenon.

11. THE EFFECTS OF CERTAIN STEROIDS—INTRAMUSCULAR AND SUBLINGUAL—ON THE BASAL BODY TEMPERATURE OF THE ADULT HUMAN MALE.

Robert M. Perlman. San Francisco, Calif.

Fifteen adult human males, exhibiting different degrees of prostatism and/or the male climacteric received various doses of androgens, estrins, and progestins, intramuscularly and sublingually, during a period of ten months. Relative effects on the earliest waking rectal temperature—or basal body temperature (B.B.T.)—were noted.

The average B.B.T. of males restored to well being with androgens was stabilized usually in the range of 98° F ±0.2° F. Massive dosage enhanced this effect. In several therapy-resistant cases, placation was obtained with extraordinary i.m. testosterone propionate doses of 150–200 mg. three times weekly.

In five cases, due to prohibitive costs of massive androgen therapy, estrins were substituted. A single dose of 2.5–5.0 mg. of equine estrogenic substances ameliorated symptoms of prostatism and, following an initial temperature lag of one to two days, depressed and maintained the B.B.T. at approximately 97^{5}° F to 97^{8}° F for one to four weeks. Penile erections were suppressed. No breast changes were observed grossly during three to four months.

Progesterone, 10–50 mg. i.m., produced B.B.T. elevations ranging from 0.1° F to 2.0° F. "Linguets" of anhydrohydroxyprogesterone acted similarly; *however*, doses 10–30 times greater, mg. for mg., were required to produce equal effects. Sensitivity to progestin fluctuated markedly from individual to individual. *In the same individual, graduated doses initiated thermal elevations directly proportional to the size of the dose.* Saturation doses gave temperature levels of 98^{4}° F to 99° F. Thermal rises persisted, in most cases, five to seven days, declining progressively, dipping, and returning to approximate starting points. *Administration of progestin resulted in intense recrudescence of prostatic and/or climacteric symptomatology.*

2:00 P.M. *Red Lacquer Room*

12. A SIMPLIFIED HYPOPHYSECTOMIZED RAT ADRENAL ASCORBIC ACID BIOASSAY METHOD FOR ADRENOCORTICOTROPIN (ACTH): SPECIFICITY AND APPLICATION TO PREPARATIVE PROBLEMS.

Paul L. Munson, Alfred G. Barry, Jr. (by invitation), and F. C. Koch. From the Pituitary Research Section, Biochemical Research Department, Armour and Company, Chicago.

The method of Sayers, Sayers, and Woodbury (*Endocrinology*, in press) for bioassay of adrenocorticotropin (ACTH) in the 24-hour-hypophysectomized rat involves com-

parison of the control ascorbic acid concentration determined in one adrenal removed just prior to intravenous administration of hormone with that of the second adrenal removed one hour later. The decrease is proportional to the amount and potency of the preparation administered. In the present modification, both adrenals are removed one hour after injection and analyzed together. This and other simplifications result in a saving of over half the assay labor without sacrifice of accuracy and have permitted application of the method to over 8000 animals. Using 20 to 30 rats for controls, Armour ACTH Standard and "unknown," the potency of the latter can be estimated with a standard error of 20 to 30 per cent.

The assay method has aided in the preparation of gonadotropic, lactogenic, thyrotropic, and growth hormone preparations essentially free from ACTH, incidentally providing evidence that these hormones do not interfere in the assay.

The recovery of the ACTH potency of a pituitary preparation in its sub-fractions, and the variations found following differing treatments to reduce oxytocic activity illustrate the usefulness of the method in ACTH preparative problems.

13. Content of Adrenocorticotropic Hormone (ACTH) in the Rat Pituitary under Optimal and Stressful Environmental Conditions.

George Sayers, Marshal Kerkin (by invitation) and J. N. Tortoreto (by invitation). From the Department of Pharmacology, University of Utah School of Medicine, Salt Lake City, Utah.

A method has been developed for the quantitative determination of the ACTH content of rat pituitaries. Lyophilized anterior pituitary tissue is extracted with 0.01 N sodium hydroxide in 0.9 per cent sodium chloride. The extracts are assayed for ACTH by the adrenal-ascorbic acid depletion method. Each pituitary (dry weight equal to one milligram) from rats kept under optimal environmental conditions has a biological activity approximately equivalent to 0.1 milligram of highly purified preparation of porcine ACTH (62AA).

Pituitaries from animals injected with histamine (10 milligrams per 100 grams of body weight) and sacrificed at the end of one hour have a biological activity equivalent to 0.07 ± 0.01 milligram of 62AA.

The adrenals of these histamine-injected animals show a depletion of ascorbic acid which can be produced by the administration of 0.05 milligram of ACTH (62AA). Thus the amount of ACTH released, as measured by the effect on adrenal ascorbic acid is largely accounted for by the loss of tropic activity from the pituitary tissue.

These data indicate that sufficiently large quantities of ACTH are stored in the pituitary to take care of the immediate requirements of the animal during the response to acute stress. Further work is in progress to determine the rate of elaboration and synthesis of this tropic hormone by the pituitary under a number of other experimental conditions.

14. The Activation of the Adrenal Cortex by Insulin Hypoglycemia.

H. Gershberg (by invitation) and C. N. H. Long. Department of Physiological Chemistry, Yale University, New Haven, Conn.

Cannon, McIver and Bliss have shown that insulin hypoglycemia causes activation of the autonomic nervous system and release of epinephrine. Since it has recently been shown that epinephrine also brings about release of the adrenotropic hormone of the pituitary and activation of the adrenal cortex, experiments have been carried out to test the hypothesis that the physiological counteractivity to insulin hypoglycemia is a

consequence of the combined action of both components of the adrenal gland. Rats were injected with 1 unit of insulin per kilo of body weight and the adrenal ascorbic acid and blood glucose were determined 1 hour later. It was found that if the blood glucose fell below 40 mg. per cent, there also occurred a large decrease in adrenal ascorbic acid, an effect which is now known to be due to the release of adrenotropic hormone.

However, if glucose is given along with the insulin to prevent a fall in the blood glucose, then no loss of adrenal ascorbic acid was observed. That it is not the level of blood glucose itself but the release of epinephrine that determines the activity of the pituitary is shown by the fact that the administration of glucose does not prevent the activation of the pituitary in such situations as exposure to cold or trauma, where the release of epinephrine is due to cause other than hypoglycemia.

15. Influence of Adenotropic Hormone on Sodium Excretion in Hypophysectomized Rats.

Betty L. Rubin and Ralph I. Dorfman. From the Departments of Biochemistry and Medicine, Western Reserve University School of Medicine and Lakeside Hospital, Cleveland 6, Ohio.

The influence of adrenotropic hormone on sodium excretion in hypophysectomized male rats has been studied using a method previously described (Dorfman, Potts, and Feil; *Endocrinology* 41: 464, 1947). This method employs radiosodium and has been shown to detect as little as one microgram of desoxycorticosterone.

Two preparations of adrenotropic hormone have been used. A highly purified preparation, kindly supplied by Dr. C. H. Li, produced statistically significant increases in sodium excretion during a six-hour test period. Under similar conditions a crude preparation, kindly supplied by Dr. D. A. McGinty, gave less consistent results, but sodium retention was indicated. The significance of these findings will be discussed.

16. Factors Influencing the Corticotropin Production of the Anterior Pituitary.

Hans Selye. From the Institut de Medecine et de Chirurgie experimentales, Universite de Montreal, Montreal, Canada.

Earlier experiments lead us to believe that under the influence of nonspecific stress (e.g., cold, infections, intoxications, nervous stimuli), corticotropin production by the pituitary is increased and this secondarily causes an excessive production of corticoids (to raise the resistance against nonspecific stress) as part of the "General-Adaptation-Syndrome." Sometimes, such prolonged stress also leads to hypertension and nephrosclerosis especially in animals sensitized by unilateral nephrectomy and diets rich in sodium and protein. It was striking, however, that while anterior-pituitary preparations or synthetic corticoids (e.g., desoxycorticosterone acetate) regularly cause nephrosclerosis and hypertension in animals so sensitized, stress (e.g., cold) did so only in a comparatively small percentage of cases. In clinical medicine stress appears to be a factor in the production of nephrosclerosis and hypertension, yet, usually in patients exposed to the continuous stress of wasting diseases, hypertension does not ensue and the adrenal cortex is not always unusually large.

Recent experiments in the rat have thrown some light upon these questions; they indicate that in the rat, stress (e.g., cold) causes marked adrenal cortical enlargement only when the protein intake is high (30% casein diets) and not when it is comparatively low (15% casein diets). A synthetic diet containing protein hydrolysates or essential amino-acids (in a quantity equivalent in nitrogen to a 30% casein diet) permits the same stress-hypertrophy of the adrenal as the high-protein diet. Apparently, amino-acids

are necessary for the normal corticotropin production during stress. It must be pointed out that high-protein diets in themselves did not cause adrenal enlargement, nephrosclerosis and hypertension under our experimental conditions so that apparently both protein catabolism and stress are necessary in order to insure optimal corticotropin production. In rats in which sudden protein catabolism is elicited by starvation, thyroxin, cold and other stresses, the adrenal cortex undergoes marked enlargement, especially if the animals were previously kept on high-protein diets and thus allowed to store protein. Conversely, after prolonged feeding on low-protein diets the adrenal response to stress is slight.

These observations suggest that in clinical medicine, hypertrophy of the adrenal and the subsequent development of "diseases of adaptation" due to corticoid intoxication may likewise depend upon simultaneous changes in protein metabolism induced by the stress.

17. The Use of Adrenocorticotropin as a Test of Adrenal Cortical Reserve.

George W. Thorn, Peter H. Forsham (by invitation), Lillian Recant (by invitation), and A. Gorman Hills (by invitation). Department of Medicine, Harvard Medical School, and the Medical Clinic, Peter Bent Brigham Hospital, Boston.

In man ACTH has been shown to be capable of stimulating the secretion of all known types of physiologically active adrenal cortical steroids. In a large group of normal subjects and patients without evidence of adrenal cortical insufficiency a fall in circulating eosinophils and a rise in urinary uric acid/creatinine ratio (the change exceeding 50 per cent in both instances) was observed following a single dose of 25 mg. of ACTH injected intramuscularly. Thirty-five patients with classical Addison's disease showed a small or insignificant fall in circulating eosinophils (mean value 6 per cent) and a rise in urinary uric acid/creatinine ratio (mean value 24 per cent). There was no overlap between the control group and the Addisonian group with respect to the changes in circulating eosinophils. There was some overlap between the maximal increase in uric acid/creatinine ratio observed in the less severe Addisonian patients and the poorest response of apparently non-Addisonian patients. A group of patients, complaining of weakness, fatigue, and hypotension without other evidences of adrenal cortical insufficiency and with evident psychological and emotional disturbances, showed an excellent response to the ACTH test dose. Hence primary adrenal cortical insufficiency could be readily excluded in this group by a relatively simple test which required only four to six hours for completion. Eight patients with evidence of anterior pituitary deficiency failed to show an entirely normal response to a single test dose of ACTH but did show some response to a forty-eight hour period of continuous administration, i.e. 10 mg. every six hours.

Patients with Cushing's disease revealed an extremely low initial level of circulating eosinophils (2 to 20 cells per cu. mm.) in contrast to normal subjects (100 to 300 cells per cu. mm.). The initial fasting urinary uric acid/creatinine ratio was similar to the values obtained in normals following ACTH administration.

18. Observations on the Pituitary-Adrenal Response Following Epinephrine Infusion in Man.

Lillian Recant (by invitation), Peter H. Forsham (by invitation), and George W. Thorn. Department of Medicine, Harvard Medical School, and the Medical Clinic, Peter Bent Brigham Hospital, Boston.

Long and his co-workers have demonstrated the response of the adrenal cortex to the administration of epinephrine to rats with an intact anterior pituitary. In previous

studies the authors have demonstrated that a sudden increase in 11–17-adrenal oxysteroids is followed by a rapid fall in circulating eosinophils in both man and rats under control conditions.

Employing the fall in circulating eosinophils as an index of adrenal steroid response, normal subjects, patients with Addison's disease, and patients with pituitary insufficiency have been investigated following the intravenous administration of 1.5 mg. of epinephrine hydrochloride in 200 cc. of saline over a one-hour period. Normal subjects and patients without evidence of adrenal or pituitary insufficiency show a maximal drop in eosinophils of 70 to 80 per cent in four hours and a maximal lymphocyte fall of 30 per cent in two hours. Patients with pituitary insufficiency show practically no change in circulating eosinophils. Patients with Addison's disease show a definite but variable response which correlates approximately with the severity of the Addison's disease clinically. These studies suggest that in man the secretion of pituitary ACTH is stimulated by epinephrine and that it is possible by this technique to differentiate between primary adrenal and pituitary-adrenal insufficiency. Studies in rats confirm these observations.

19. Fate and Metabolic Action of Intravenously Administered Adrenocorticotropic Hormone (ACTH).

Thomas W. Burns (by invitation), George Sayers, Frank H. Tyler (by invitation), B. V. Jager (by invitation), T. B. Schwartz (by invitation), Emil L. Smith (by invitation) and L. T. Samuels. From the Departments of Pharmacology, Medicine and Biochemistry, University of Utah School of Medicine, Salt Lake City, Utah.

A normal male subject was infused with 200 ml. of 0.85 per cent sodium chloride containing 240 mg. of A.C.T.H. (Armour's 37-KE, containing 0.005 units of pressor activity per mg.) over a period of 50 minutes. Except for a mild chill which lasted 25 minutes no untoward reactions occurred. Blood pressure remained within normal limits except for a slight rise during the course of the chill. At the end of the infusion the plasma level of A.C.T.H. (as bioassayed by the adrenal-ascorbic acid depletion method) was approximately 3000 micrograms per 100 ml. plasma; within the first hour after infusion the level had dropped to 200 micrograms per 100 ml., and at the end of 5 hours was about 25 micrograms per 100 ml. There was no detectable activity in the plasma at the end of 24 hours. Less than 200 micrograms was excreted in the urine during this 24-hour period.

Following hormone administration, urinary 17-ketosteroids and neutral-lipide reducing substances (Heard, Sobel and Venning) were elevated. A transitory mild elevation in the fasting blood glucose occurred. A diabetic type of response was obtained from a meal ingested 6 hours after hormone administration. The level of serum phosphate fell, suggesting glycogen storage. These changes occurred concomitantly with an increase in urinary nitrogen excretion. The serum uric acid level remained unchanged while uric acid excretion was increased. There was a slight but apparently significant increase in the creatinine clearance. Circulating eosinophiles and lymphocytes were greatly reduced in number. No changes took place in plasma albumin and gamma globulins as determined by salt fractionation and electrophoretic methods. Anti-streptolysin "O" and anti-typhoid antibodies failed to rise. Certain serum peptidases were elevated. Blood pH, serially determined, reached a maximum level of 7.60. All of the above metabolic changes reached a maximum and had returned to normal within the first 12 hours after A.C.T.H. administration.

20. Metabolic Changes Following the Administration of Pituitary Adrenocorticotropic Hormone (A.C.T.H.) to Normal Humans.

H. T. McAlpine (by invitation), E. H. Venning, L. Johnson (by invitation), V. Schenker (by invitation), M. M. Hoffman and J. S. L. Browne. McGill University Clinic, Royal Victoria Hospital, Montreal, Quebec.

Massive doses (240 to 420 mg.)* of adrenocorticotropic hormone (Armour) were given to two normal males and one normal female in divided doses over a twenty-four hour period. The subjects were maintained on a constant diet for a control period and throughout the experiment. Following the administration of the hormone there was a decreased urinary excretion of sodium and chloride and an increased excretion of potassium, uric acid and nitrogen. This was followed by a sodium and chloride diuresis and a decreased excretion of potassium. In addition, following the A.C.T.H. administration there was a striking urinary excretion of glucose, varying from 12 to 30 grams. In spite of this glycosuria there was a marked reduction in the urine volume (in one case to 600 cc. per day). There was also a moderate elevation of the blood sugar. The slight increase in nitrogen excretion was insufficient to account for the amount of glucose excreted. The significance of these findings with regard to the pituitary-adenal relationship will be discussed.

21. The Effect of Adrenocorticotropin on Antibody Levels in Normal Human Subjects.

P. H. Herbert and J. A. de Vries (introduced by J. S. L. Browne). McGill University Clinic, Royal Victoria Hospital and Department of Bacteriology and Immunology, McGill University, Montreal.

Subjects were immunized with a commercial preparation of staphylococcal toxoid and later received corticotropin. Before the experiments were initiated the anti-hemolysin titres were allowed to fall well below the maximum previously attained. Figures for antibody levels before and after injecting the hormone are presented, together with total, eosinophil and differential leucocyte counts and plasma protein and hematocrit values.

The quantities of hormone injected ranged from 40 to 420 mg. (equivalent to 28 to 210 mg. of the Armour standard preparation LA-1-A) the smaller amounts being given in a single injection and the larger in a series of injections over periods varying from one hour to twenty-four hours.

In no subject was there a significant rise in antibody level after giving corticotropin even though the larger doses were sufficient to cause a great increase in the excretion of urinary corticoids. In all subjects there was a fall in the lymphocyte count, the least being 25% and the greatest 68% of the initial control value. These lymphocyte counts returned quickly to normal and the extent of the fall was not related to the amount of hormone given. The eosinophil counts fell more quickly than the lymphocyte counts and the decrease was greater with the larger amounts of hormone. In experiments in which injections were continued for the longer periods these cells almost disappeared from the blood. A fall in hematocrit of 10–17% suggested an increase of blood volume but the plasma proteins in most cases tended to maintain the initial levels.

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* These amounts are equivalent to 120 to 210 mg. of the Armour standard preparation LA-1-A.

22. A Comparison of the Effect on Bone Formation of the Hyperadrenocorticism of Cushing's Syndrome with That Induced by Adrenocorticotropic Hormone (A.C.T.H.).*

Frederic C. Bartter, Anne P. Forbes and Fuller Albright. Massachusetts General Hospital, Boston, Mass.

One of the cardinal features of Cushing's syndrome is osteoporosis. By definition, osteoporosis is that form of demineralization which is due to decreased formation of bone matrix by osteoblasts. The serum alkaline phosphatase, an index of osteoblastic activity, is low. It is thus a disorder of protein metabolism, not of calcium metabolism. However, one cannot deposit calcium without matrix. It follows that the development of osteoporosis will be accompanied by an increased excretion of calcium if bone destruction remains constant.

The data to be presented are taken from metabolic balance studies on patients with panhypopituitarism, acromegaly, osteitis deformans, and ovarian agenesis. The administration of A.C.T.H. produced a marked increase in urinary calcium excretion in all patients, save two who received stilbestrol throughout the study. In the three patients in whom the serum phosphatase was initially elevated, A.C.T.H. produced a fall; in two of these the hypercalcuria was accentuated.

These studies support the hypothesis that A.C.T.H. causes elaboration of an adrenal cortical hormone which inhibits the production of bone matrix by osteoblasts.

23. Adrenal Cortical Unresponsiveness in Patients with Gastric Cancer.

Edward C. Reifenstein, Jr., N. F. Young, Aurelia Potor, Benedict Duffy and F. Homburger. Department of Clinical Investigation of the Sloan-Kettering Institute, Memorial Cancer Center, New York.

Persons with normally responsive adrenal cortices respond to the injection of anterior pituitary adrenocorticotropic hormone with a fall in the blood eosinophil level and a rise in the urinary uric acid/creatinine ratio. In contrast, the majority of patients with gastric cancer respond to injection of this hormone preparation only with the fall in the eosinophil level. Studies are in progress to determine the nature of the lack of response to pituitary tropic hormone stimulation of the adrenal cortical mechanism for regulating the uric acid/creatinine ratio in patients with gastric cancer.

Previous studies have shown that the administration of adrenal cortical extract is necessary to induce normal liver glycogen deposition in patients with gastric cancer. The effect of the administration of adrenocorticotropic hormone on liver glycogen deposition in patients with gastric neoplasms is being studied to see whether in patients with this disease the adrenal cortical mechanism involved in glycogen deposition is also unresponsive to pituitary tropic hormone stimulation.

It remains to be determined whether the adrenal cortex does not respond to pituitary tropic hormone stimulation because it is less sensitive or because it is functioning to the limit of its capacity.

24. The Excretion of Adrenal Metabolites in Human Urine.

Konrad Dobriner, Seymour Lieberman and C. P. Rhoads. Sloan-Kettering Institute for Cancer Research, New York.

Androsterone and 11-hydroxy androsterone have been recognized as normal constituents in the urine of males and females. In the course of hydrolysis 11β-hydroxy andros-

* The A.C.T.H. was furnished by Dr. John Mote of the Armour Laboratories and by Dr. Choh Hao Li of the University of California.